JOHN ELIAS BALDACCI
GOVERNOR

STATE OF MAINE
MAINE DEPARTMENT OF AGRICULTURE, FOOD & RURAL RESOURCES
BOARD OF PESTICIDES CONTROL
28 STATE HOUSE STATION
AUGUSTA, MAINE 04333-0028

SETH H. BRADSTREET III
COMMISSIONER
HENRY JENNINGS.
ACTING DIRECTOR

TO: Maine Board of Pesticides Control
FROM: Lebelle Hicks PhD DABT
RE: Background documents for the Dylox public hearing

November 13, 2006

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Attached are three documents regarding the Board's review activities on the pesticide trichlorfon, the active ingredient in Dylox products.

1. July 18, 2005; Conclusion of the MAC's Trichlorfon and Comparative risks from grub control agents

2. December 20, 2004; Draft of the Medical Advisory Committee's (MAC) Trichlorfon Review

3. June 29, 2005; Grub and Chafer Control, Maine BPC Medical Advisory Committee report

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ROBERT W. SPEAR
COMMISSIONER
ROBERT I. BATTEESE, JR.
DIRECTOR

TO: Board Members
FROM: Lebelle Hicks PhD DABT
RE: MAC Review of Trichlorfon (Dylox) and Comparative risks from other Grub Control Agents

July 18, 2005

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At their June 29th MAC meeting the review of trichlorfon was completed and the MAC members also discussed the relative risks of the six products which could be used in a grub/chafer control program. Five of the six MAC members were present, and their conclusions regarding trichlorfon were:

> "Trichlorfon, while used extensively as a general use (available to homeowners) product in lawn care in other states, remains of concern to our Medical Advisory Committee due to issues of acute and chronic toxicity. EPA appears to be phasing out many organophosphates due to acute toxicity to applicators (professional and homeowners) and public concerns about food and water safety. It is allowing the use of trichlorfon on turf because the only remaining food use is as a cattle dip in another country. EPA does not appear to be concerned about most exposure scenarios or surface water levels being exceeded. But the MAC has concerns that the substance may be carcinogenic at high doses and may be acutely toxic to lawn care workers, homeowner-applicators, and those who use the turf immediately after application. EPA's estimates of exposure and risks do not indicate that the "high" exposures are occurring with lawn care uses. However, the MAC cannot recommend trichlorfon use as a first line agent or as a general use product available to homeowners. The committee encourages further research on biological controls. It leaves to the Board to change the regulatory status of this product."

Attached is a summary of the comparative risks of the six grub/chafer control agents. I will be at the meeting to address questions.

Trichlorfon; Background

Trichlorfon (trade name Dylox) was added to the Maine state restricted use list in Chapter 40 in 1981 based on concerns of mutagenicity. At the request of the registrant and blueberry growers, it was moved to the state limited use list in the mid 1980s and a permitting system was developed for controlling blueberry span worm. In June of 1992, the Medical Advisory Committee (MAC) evaluated trichlorfon, unclear results and the MAC decided not to recommend any change in status for trichlorfon. Instead the MAC agreed to re-evaluate trichlorfon on the conclusion of the Environmental Protection Agency's (EPA) organophosphate risk assessment (1).

The MAC reviewed EPA's findings on trichlorfon at a February 19, 2004 meeting but still wished to see a comparison of trichlorfon with alternative grub/chafer control products. Their request was explained at the March 19, 2004 Board meeting where the Board members agreed the comparison would help put trichlorfon in perspective with other available insecticides. There are many grub/chafer control products registered nationally. Six of these active ingredients were identified by UMaine Cooperative Extension for grub/chafer control in Maine. The chemistry, efficacy and registration status for these six grub control agents: carbaryl, halofenozide, imidacloprid, permethrin, thiamethoxam and trichlorfon are summarized in Table 1 (2.).

The Food Quality Protection Act (FQPA) was passed in 1996 and the standard of care for dietary exposure to pesticides was changed, primarily where children are concerned. EPA was charged with re-assessing dietary tolerances for the most risky classes of pesticide compounds first. Because of this, tolerance reassessment has been done for trichlorfon and carbaryl. EPA has not prepared Final Registration Eligibility Documents (REDs) for these two compounds. EPA's Health Effects Division has released memos detailing their assessments and an "Interim Report on Tolerance Reassessment for Trichlorfon" from 2001 and the "Carbaryl Interim Re-registration Eligibility Document" from 2003. Since all food uses but one on imported beef have been canceled for trichlorfon, EPA has concluded the remaining risks are sufficiently low so that trichlorfon may continue to be sold as a general use pesticide for ornamental turf and residential lawn care applications.

In addition, there are FQPA level tolerance assessments for thiamethoxam and imidacloprid because these are newer agents and petitions for new food tolerances since 1996 have been granted. There are no food uses for halofenozide and the permethrin re-assessment is due in 2006 (2). As seen in Table 2., the amounts and types of EPA risk assessment data for these two compounds is lacking.

Risk Assessment

In order to evaluate the comparison of the risks from the grub control products, background on EPA's risk assessment policies and procedures is in order. Risk is mathematically equal to the toxicity factor times the exposure factor. A low toxicity product with high exposure could have the same risk as a highly toxic product with low exposure. Risk assessments generally take one

of three forms depending on the exposure pattern:

(1) for diet and drinking water exposures, the reference dose (RfD) is used;
(2), for both professional and home owner applicators, the Margin of Exposure (MOE) approach is used and
(3) for carcinogens with an adequate dose tumor response relationship the linear multistage model is utilized **(this method was not used for tolerance assessment for any of the six grub control products because the expected exposure is short term/intermediate and non-cancer risks are the drivers for the assessments)**.

In the first two instances, the toxicity factor is the No Observable Effect Level (NOAEL) or Low Observable Effect Level (LOAEL) if the NOAEL is not available. The exposure factor is calculated based on exposure studies. Uncertainty factors (UF) ranging from 100 to 1000 are used depending on the data set. The specific UF are 10 for extrapolating from animals to people; 10 for variability in the human population and 3 to 10 for an incomplete database.

Mathematically the RfD is equal to NOAEL/UF (equation a). When using a RfD methodology, the uncertainty is built into the equation. Exposures are then summed and compared to the RfD. If the RfD is exceeded, then changes in exposure such as cancellation of uses; reduction in use rates; increase in treatment intervals and/or increases in pre-harvest intervals occur.

With the MOE methodology the MOE is equal to the NOAEL/exposure dose (equation b). In this case, EPA's acceptable risk level is driven by the toxicity database and/or population exposed. Different risk acceptable risk levels are used in cases where:

- a LOAEL is being used instead of a NOAEL,
- there is a chronic exposure and some evidence of carcinogenicity,
- children, as a sensitive sub-population are being exposed, or
- the exposure is occupational.

The Food Quality Protection Act Safety Factor (FQPA SF) is applied to the RfD or the MOE to assess risk when children are likely to be exposed (diet, drinking water, post application residential exposures). The Population Adjusted Doses (PAD) = Reference Dose (RfD) divided by the Food Quality Protection Act Safety Factor (FQPA SF) (equation c). If the FQPA SF is equal to 1 (no evidence of increased sensitivity in developing fetuses, then the RfD is equal to the PAD (2).

Equation a: $\frac{\text{NOAEL}}{\text{UF}} = \text{RfD}$ Equation b: $\frac{\text{NOAEL}}{\text{Exposure dose}} = \text{MOE}$

Equation c: $\frac{\text{RfD}}{\text{FQPA SF}} = \text{PAD}$

While dietary exposure is not the issue here, the information presented in Table 2., will provide a

basis for comparing the active ingredients (2). The acute toxicity risk assessment, establishing the aRfD, was done using data from the acute neurotoxicity (imidacloprid, thiamethoxam and trichlorfon) and the developmental neurotoxicity studies (carbaryl) in rats. With regard to developmental and reproductive toxicity, two materials have retained FQPA SF of 10 (thiamethoxam and trichlorfon) and the other two have been reduced to 1 (carbaryl and imidacloprid). The cancer rankings are also varied, one not likely (imidacloprid), one suggestive (permethrin), one likely at high doses, not likely at low doses (trichlorfon) and two likely (carbaryl and thiamethoxam). Here again no data for halofenozide was identified or reviewed (2).

The FQPA level non-food risk assessments for ornamental turf and lawn care for carbaryl and trichlorfon utilizing MOE methodology summarized in Table 3. (2). EPA's acceptable risk level for these MOEs is > 1000 for toddlers and > 100 exposed to trichlorfon and 100 for all carbaryl short and intermediate term exposures (post application on lawns and golf courses). As seen in Table 3., the trichlorfon MOEs are all greater than the acceptable risk levels while the carbaryl MOEs are below the acceptable risk level for homeowners applying carbaryl (2 of the 4 scenarios) and 2 of the 3 post application scenarios. The foot note explains that EPA is currently reviewing a Bayer refined exposure assessment to determine if these uses will remain on the carbaryl labels.

The role of these products in grub/chafer control in New England has been evaluated by Patricia Vittum PhD of the University of Massachusetts Cooperative Extension as shown in Attachment 1. (3). Please note that while carbaryl made the list from UMaine extension, it is not recommended in the UMass fact sheet on controlling grubs in New England.

Trichlorfon Review

Maine Board of Pesticides Control; Medical Advisory Committee

Lebelle Hicks PhD DABT
December 20, 2004

ACRONYMS	
A	Applicator
AChE	Acetyl Cholinesterase
AD	Alzheimer's Disease
aPAD	Acute Population Adjusted Dose
aRfD	Acute Reference Dose
BEAD	Biological and Economic Division
BPC	Board of Pesticides Control
CARC	Cancer Assessment Review Committee
ChE	Cholinesterase
CPRC	Cancer Peer Review Committee
cPAD	Chronic Population Adjusted Dose
cRfD	Chronic Reference Dose
CSF	Cerebral Spinal Fluid
CSFII	Consumer Survey of Food Intake in Individuals
DDVP	Dichlorvos
DEEM	Dietary Exposure Evaluation Model
DNT	Developmental Neurotoxicity Study
DWLOC	Drinking Water Level of Concern
EEC	Expected Environmental Concentration
EPA	US Environmental Protection Agency
FAO	Food and Agriculture Organization
FIFRA	Federal Insecticide, Fungicide Rodenticide Act
FQPA	Food Quality Protection Act
FQPA SF	Food Quality Protection Act Safety Factor [1 to 10]
GD	Gestational Day

ACRONYMS	
GLN	Guideline Number
HDT	Highest Dose Tested
HED	Health Effects Division
L	Loader
LC_{50}	Lethal median Concentration
LD_{50}	Lethal median Dose
LD	Lactational Day
LOAEL	Lowest Observable Adverse Effect Level
LOC	Level of Concern
M	Mixer
MAC	Medical Advisory Committee to the Board of Pesticides Control
MCL	Mononuclear Leukemia
MCV	Mean Cell Volume
MOE	Margin of Exposure = No Observable Adverse Effect Level divided by Exposure *The higher the MOE the less the risk.*
MRID	EPA's Master Record Identification Number
MSDS	Material Safety Data Sheet
MTD	Maximum Tolerated Dose
NA	Not Applicable
ND	No Data
NOAEL	No Observable Adverse Effect Level
LOAEL	Lowest Observable Effect Level
NR	Not Reported
ns	not statistically significant
NTP	National Toxicology Program
OPIDN	Organophosphate Induced Delayed Neuropathy

ACRONYMS	
OPP	Office of Pesticides Programs
ORETF	Outdoor Residential Exposure Task Force
PAD	Population Adjusted Dose; Reference Dose divided by the FQPA Safety Factor
PND	Post Natal Day
PPE	Personal Protective Equipment
RBC	Red Blood Cells
RED	Registration Eligibility Document
RfD	Reference Dose = NOAEL divided by Uncertainty factors
S9	Mammalian liver enzyme fraction used in *in vitro* mutagenicity studies
SAP	Scientific Advisory Panel
SLU	State Limited Use
SRU	State Restricted Use
T ½	Half life
TRED	EPA (2001) "Report on FQPA Tolerance Reassessment Progress and Interim Risk Management Decision for trichlorfon"
UF	Uncertainty Factors; usually a factor of 10 for intraspecies extrapolation; 10 for interspecies extrapolation; 10 for using a LOAEL instead of a NOAEL and 3 or 10 based on cancer classification (not quantifiable for cancer risk assessment)
WHO	World Health Organization

LIST OF APPENDICES

BACKGROUND

Trichlorfon (trade name Dylox) was added to the Maine state restricted use list, Chapter 40, in 1981 based on concerns of mutagenicity (1). At the request of the registrant, it was moved to the state limited use list in the mid 1980s and a permitting system was developed for controlling blueberry span worm. In June of 1992, the Medical Advisory Committee (MAC) evaluated trichlorfon (2), and because it is a dichlorvos (DDVP) releasing product, the MAC did a brief survey of information on all three DDVP forming products; trichlorfon, naled and DDVP (3). The results were unclear and the MAC decided not to recommend any change in status for trichlorfon. Instead the MAC agreed to re-evaluate trichlorfon on the conclusion of the Environmental Protection Agency's (EPA) organophosphate risk assessment.

With the passage of the Food Quality Protection Act in 1996, EPA reassessed all of the organophosphates including trichlorfon and DDVP. Registration Eligibility Documents (RED) for these two compounds have not been written for these two compounds. However, memos from EPA's Health Effects Division (HED) risk assessment groups are available (4, 5, 6) along with the "Interim Report on Tolerance Reassessment for Trichlorfon" (7).

Trichlorfon (drug name: metrifonate, CAS # 52-68-6) is an organophosphate insecticide with a molecular weight of 257.4. It is soluble in water (120 g/L @ 20°C), non-enzymatically forms DDVP under mildly basic conditions and acts as a slow release formulation of DDVP in biological systems (9). With these characteristics in mind, the acute and subchronic toxicity studies, neurotoxicity, developmental, reproductive, carcinogenicity and genotoxicity sections of the DDVP toxicity database are discussed below. In the sections addressing the pesticide issues, the compound is referred to as trichlorfon and in the clinical section it is referred to metrifonate.

Registration and Recent Use History in Maine

There are currently 4 products containing trichlorfon registered in Maine (SPIRS 2003). They are Dylox 80 Turf (EPA # 3125-184) and Dylox 6.2 Granular (EPA # 3125- 406 (4 distributers)), Dylox 6.2 G (EPA # 2125-507) and Trichlorfon 6.2 Granular (EPA #32802-29). Bayer Environmental Science is the basic producer and major registrant for trichlorfon products (8).

The Dylox 80 label has the signal word "WARNING" and is EPA toxicity category II (EPA's Toxicity Categories and signal word classification schemes are found in Appendix A). The label also restricts its sale and use to commercial applicators, and provides directions for using a mask or respirator during mixing and loading, and to wear goggles or safety glasses. The directions also call for mixing quantities of 100 gallons, indicating a professional use product.

The Dylox 6.2 granular products have the signal word "CAUTION"and are in EPA's toxicity category III or IV. The labels contain warnings to not breathe the dust, avoid contact with eyes, skin and clothing, and to keep children and pets off of treated lawns until dry. Their use

directions specify using a spreader at the rate of 2 lbs per 1000 sq ft, making them obvious homeowner products.

TOXICOLOGY

Acute Studies; Trichlorfon

EPA considers the registration database for trichlorfon complete (5). Trichlorfon is a moderately toxic organophosphate which releases the direct acting agent DDVP. As with the group of organophosphate insecticides, cholinesterase (ChE) inhibition is the mechanism of action (9). The acute animal toxicity studies in the trichlorfon registration database and EPA toxicity categories for technical trichlorfon are summarized in Table 1.

Table 1. Acute Toxicity of Technical Grade Trichlorfon (5).

Study Route Species	Results	EPA Toxicity Category [(a)]
Acute Oral rat	LD_{50} = 136 to 173 mg/kg	II
Acute Dermal rabbit	LD_{50} = 2,000 mg/kg	III
Acute Inhalation Rat	LC_{50} = 533 mg/m^3	III
Acute Eye Irritation Rabbit	Moderately irritating	II
Acute Dermal Irritation	Non-irritating	IV
Skin Sensitization	Moderate contact allergen	NA

(a) See Appendix A for description of EPA Toxicity Categories

In the acute neurotoxicity study, Fischer 344 rats were administered trichlorfon at doses of 0, 10, 15 or 200 mg/kg by the oral route. Endpoints measured included Functional Observational Battery (FOB), motor activity and ChE activity. The No Observable Adverse Effect Level (NOAEL) for neurological endpoints was 10 mg/kg and the Lowest Observable Adverse Effect Level (LOAEL) was 50 mg/kg based on clinical signs, oral, nasal and urine stains, alterations in FOB, decreased motor activity and brain ChE inhibition (5).

EPA reported a human NOAEL of 2.5 mg/kg from a single dose clinical study. The LOAEL from this study was 5.0 mg/kg based on inhibition of plasma and RBC and clinical signs of nausea, vomiting and diarrhea (5).

Acute Studies; DDVP

EPA published a revised preliminary HED risk assessment for DDVP in August of 2000. While EPA considers the toxicological database for DDVP essentially complete, the full RED is not currently available. The EPA acute toxicity guideline studies for DDVP are summarized in Table 2. (6).

DDVP was negative for OPIDN in hens. In the acute neurotoxicity in rats the NOAELs was 0.5 mg/kg and the LOAEL was 35 mg/kg as evidenced by changes in FOB and motor activity. There was no neuropathology found in this study (6).

Table 2. Acute Toxicity of Technical Grade DDVP (6).

Study Route Species	Results	EPA Toxicity Category [(a)]
Acute Oral rat	LD_{50} = 80 mg/kg (M) 56 mg/kg (F)	II
Acute Dermal rabbit	LD_{50} = 107 mg/kg (M) 75 mg/kg (F)	I
Acute Inhalation Rat	LC_{50} = > 0.198 mg/L	II
Acute Eye Irritation Rabbit	Mild irritant	III
Acute Dermal Irritation	Mild irritant	IV
Skin Sensitization	No study Available	NA

(b) See Appendix A for description of EPA Toxicity Categories

Subchronic Studies; Trichlorfon

In the 90 day neurotoxicity study in hens the dose levels were 0, 3, 9 or 18 mg/kg/day. No overt signs of Organophosphate Induced Delayed Neuropathy (OPIDN) were observed. There was a histological finding described as axonal degeneration at the highest dose tested (HDT) and NOAEL was determined as 9 mg/kg/day (5).

Fischer 344 rats received trichlorfon in their diets at concentrations of 0, 100, 500 and 2500 ppm (equivalent to 0, 6, 31, 165 mg/kg/day for males and 0, 7, 35, and 189 mg/kg/day in females) for 90 days. The ChE NOAEL was 100 ppm (6 mg/kg/day) and the LOAEL was 500 ppm (165 mg/kg/day) based on plasma, RBC and Brain ChE inhibition. The systemic and neurotoxicity NOAEL was 100 ppm (31 mg/kg/day) and the LOAEL 500 ppm (165 mg/kg/day) based on clinical signs during the FOB, uncoordinated righting reflex in males, reduced motor and locomotor activity in males and females. The neurological endpoints were minimal myelin degeneration of the spinal nerve routes in both sexes (5).

Subchronic Studies; DDVP

A similar set of subchronic animal studies have been performed using DDVP. The results of the 28-day hen study for DDVP were negative for neuropathology. The NOAEL for ChE inhibition was 0.1 mg/kg/day with a LOAEL of 0.3 mg/kg/day for brain ChE inhibition (6).

The most sensitive endpoint in the 90-day rat toxicity study was ChE inhibition. The NOAEL was 0.1 mg/kg/day and the LOAEL 1.5 mg/kg/day with observations of plasma and RBC inhibition (6).

In the 90-day rat neurotoxicity study the NOAEL was 0.05 mg/kg/day with a LOAEL of 1.0 mg/kg/day based on plasma and RBC ChE inhibition in males and females and brain ChE inhibition in males (6).

Chronic /Cancer Studies; Trichlorfon

Chronic Studies

The chronic studies for trichlorfon are summarized in Table 3. EPA has chosen the 10 year monkey study as the basis of the chronic Reference dose (RfD).

Table 3. Trichlorfon Chronic Toxicity; Non-cancer endpoints				
Study		NOAEL	LOAEL	Effects at LOAEL
Chronic Rat Diet ♂; 0, 4.4, 13.3, 75 mg/kg/day: ♀; 5.8, 17.4. 93.7 mg/kg/day (EPA 1999a)				
Systemic	Male	4.4	13.3	↑ in renal calcification ↑ in hypercholesteroemia
	Female	5.8	17.4	↑ in hypercholesteroemia
ChE inhibition	Male	4.4	13.3	RBC
	Female	5.8	17.4	
	Male	4.4	13.3	Brain
	Female	5.8	17.4	
Chronic Dog Diet 0, 1.2, 6.3, 12.5 and 25 mg/kg/day [(a)] (EPA 1999a)				
ChE inhibition		6.3	12.5	RBC
Chronic Monkey Oral in and Orange drink; 0, 0.2, 1.0, 5.0 mg/kg/day (EPA 1999a)				
ChE inhibition	Male	ND	0.2	RBC
	Female	0.2	1.0	
	Male	0.2	1.0	Brain
	Female	ND	0.2	
	Male	0.2	1.0	Plasma
	Female	ND	0.2	

Table 3. Trichlorfon Chronic Toxicity; Non-cancer endpoints				
Study		NOAEL	LOAEL	Effects at LOAEL
Chronic Mice Diet 0.45, 135, 405 mg/kg/day (EPA 1999a)				
Systemic		ND	45	Clinical signs
ChE inhibition	Female	ND	45	Plasma
	Male		45	RBC
	Male		45	Brain

Carcinogenicity Findings for Chronic Studies

The evaluation of the potential for exposure to trichlorfon to result in chronic disease and an increase in human cancer is a very complex process. It includes evaluation of data from five chronic bioassays, mutagenicity studies for trichlorfon, as well as, similar data for the active metabolite DDVP. EPA's current guidelines for chronic bioassays require that the HDT result in systemic toxicity and that the maximum tolerated dose (MTD) is not exceeded. At the MTD survival of the animals is not compromised and the major detoxification pathway is not saturated (16). In addition, the validity of the studies is judged, and those not meeting current research standards are discarded. EPA ranks the studies as:

1.) Guideline: study meets the specific data need for an individual regulatory guideline,

2.) Supplemental: study provides some useful information, but is scientifically deficient in some area, or

3.) Invalid: study is flawed to the point of not providing useful data (16).

In the discussion below, the studies reported are Guideline unless specified. Invalid studies were not included in the EPA review.

Monkey
Rhesus monkeys received trichlorfon in an orange drink for 10 years. The dose levels were 0.0.2, 1.0 and 5 mg/kg/day for 6 days a week. The LOAEL was 0.2 mg/kg/day based on a decrease (39%) of plasma ChE and a decrease (22%) in brain ChE (in males only). At the HDT (5.0 mg/kg/day), effects observed included decrease in body weight (both sexes) and anemia. Transitory signs (pupil constriction, muscle fasiculations and diarrhea) of ChE inhibition were reported in females during the first month of the study (5).

Dog
In the chronic dog study, beagles received dietary doses of 0, 50, 250, 500, or 1,000 ppm (equivalent to 0, 1.2, 6.3, 12.5 or 25 mg/kg/day). The NOAEL was reported as 6.3 mg/kg/day based on decreases in plasma and RBC ChE depression at the next higher dose. At 25 mg/kg/day, spleen and lymphoid atrophy were observed. This study was ranked supplementary with the data requirement for a non-rodent chronic study filled by the monkey study described above (5).

Rat
In the chronic rat study, the dietary concentrations were 0, 100, 300, and 1,750 ppm (equivalent to 0, 4.4, 13.3 and 75 mg/kg/day in males and 0, 5.8, 17.4 and 93.7 mg/kg/day in females) for 2 years (referred to as "the full rat study"). The chronic NOAEL was 4.4 mg/kg/day based on RBC and brain ChE inhibition, as well as an increase in renal calcification in males at the 13.3

mg/kg/day dose level. At the HDT the gross findings included granular kidneys and foci in the lungs of the females. Enlarged duodenums, thickened and granular non-glandular stomachs were observed in the males. There were microscopic findings of hyperplasia of the small intestines, non-glandular gastritis in the stomach, inflamation in the lungs and chronic nephropathy and renal calcification. Decreases in body weight gain were seen in both sexes at the HDT during week 13. Affected blood parameters included lipids and iron levels (5).

In a separate 2 year study, two groups of rats were used, the control group and a dietary concentration of 2,500 ppm trichlorfon. This resulted in dose levels of 0 and 129 mg/kg/day in males and 0 or 159 mg/kg/day in females (referred to as the "single dose rat study"). The dose in this study exceeded the maximum tolerated dose (MTD). Effects observed included: a decrease in body weight and in body weight gain, increases in urine stain, rough coats, pale eyes, decreases in RBC parameters, hypercholesterolemia, increases in hepatic enzymes and decreases in plasma, RBC and brain ChE (5).

Mice

The chronic mouse study (referred to as "the mouse study") utilized CD-1 mice and dietary concentrations of 0, 300, 900 or 2700 ppm (equivalent to 0.45, 135 or 405 mg/kg/day). Clinical signs, vaginal discharges, urine staining and ear lesions were observed at all dose levels. Plasma, RBC and brain ChE levels were depressed at all dose levels (5).

Cancer Results from Chronic Studies; Trichlorfon

In the evaluation of the carcinogenicity database, historical controls from the contract labs are also used to identify common tumors in a strain of rodent (16). EPA's final evaluation and conclusions are made by two peer review committees, the Carcinogen Peer Review Committee (CPRC) and the Cancer Assessment Review Committee (CARC). Given the extensive peer review in the EPA cancer evaluation process, the data from the individual studies is presented followed by EPA's conclusions.

The results of the tumor responses from the chronic animal studies as summarized in the 1999 Health Effects Division (HED) Toxicology Chapter for Trichlorfon are summarized in Table 4.

Table 4. Tumor Responses from the Chronic Animal Studies for Trichlorfon (5)

Species (sex)	Doses mk/kg/day	Response
Monkey	0.2 to 5	Negative
Dog [(a)]	1.2 to 25	Negative
Rat (male) full study	4.4	↑ Mononuclear leukemia [(b)] (ss) [(c)]
	13.3	Negative

Table 4. Tumor Responses from the Chronic Animal Studies for Trichlorfon (5)		
Species (sex)	Doses mk/kg/day	Response
	75	↑ Mononuclear leukemia (ss), ↑ Benign pheochromocytomas [(d)]
Rat (male) single dose [(e)]	129	↑ Renal tubular adenomas (ns) [(e)] ↑ Alveolar/bronchiolar adenomas (ns)
Rat (female) full study	5.8	Negative
	17.4	Negative
	93.7	Negative
Rat (female) single dose [(b)]	159	↑ Alveolar/bronchiolar carcinomas combined (ns)
Mouse (male)	0.45	↑ Hepatocellular adenomas (ns)
	135	↑ Hepatocellular adenomas (ns)
	405	↑ Hepatocellular adenomas (ns)
Mouse (female)	0.45	↑ Alveolar/bronchiolar adenomas (ss) ↑ Alveolar/bronchiolar adenomas and carcinomas combined (ss)
	135	↑ Alveolar/bronchiolar carcinomas (ss) ↑ Alveolar/bronchiolar adenomas and carcinomas combined (ss)
	405	Negative

(a) EPA ranked supplementary,
(b) within historical control range
(c) ss = statistically significant
(d) slightly outside historical range
(e) exceed MTD
(f) ns = not statistically significant

Following their extensive peer review of the chronic bioassay, EPA's interpretation of tumor incidence from the full rat study is:

> "Under the conditions of the study, the test material was associated with an increase in the incidence of benign pheochromocytomas in high dose males which was slightly outside of the historical control range. **Since these tumors are very common in this strain of rats and were not present in the same strain at a higher dose level in another study (discussed below), they were not considered to be compound related by the Health Effects Division (HED) Carcinogenicity Peer Review Committee (CPRC)** . A statistically significant increase in the incidence of mononuclear cell leukemia was reported for low and high dose males; however, the incidence of this tumor was within the historical control range. The highest dose tested was considered by the Cancer Assessment Review Committee (CARC) as adequate based on the compound-related effects on clinical chemistry parameters, gross and microscopic pathology and clinical findings of paleness and hunched backs in males and rough hair coats in females" (5).

Likewise, EPA's evaluation of the single dose rat study is as follows:

> "Trichlorfon was associated with an increase in the incidence of alveolar/bronchiolar adenomas in males, renal tubular adenomas in males and alveolar/bronchiolar carcinomas in females. While none of these tumors were reported at statistically significant levels, the incidences were well outside of the historical control range. There was no compound related increase in the incidence of either benign pheochromocytomas or in the incidence of mononuclear cell leukemia. In this same study, administration of the test material was associated with a decrease in body weight and body weight gain (10.5% males and 18.5 % females), increased incidences of urine stain, rough coats and pale eyes, decreases in erythrocyte parameters (hematocrit, hemoglobin , RBC count and MCV), hypercholesterolemia and increases in hepatic enzymes (SAP, AST, ALT and GGT). Decreases in plasma (63% males, 52% females) and erythrocyte (38% males, 30% females) cholinesterase activity were reported in both sexes of animals when treated groups were compared to controls. Brain cholinesterase activity was 58 and 54% lower than controls for males and females, respectively. **Compound related non-neoplastic lesions included duodenal hyperplasia, gastritis, pulmonary hyperplasia and inflammation, nasolacrimal inflammation, hepatocellular hyperplasia and vacuolation, chronic nephropathy and an increased incidence of dermal lesions were all reported at 2500 ppm.** CPRC concluded that this study was conducted at a level which exceeded the maximum tolerated dose (MTD)" (5).

Finally, EPA's interpretation of the mouse study is:

> "In the low dose females, there was a statistically significant increase in the incidence of alveolar/bronchiolar adenomas and combined alveolar/bronchiolar adenomas and

carcinomas. In the mid-dose group, there was a statistically significant increase in the incidence of alveolar/bronchiolar carcinomas and combined alveolar/bronchiolar adenomas and carcinomas. No significant differences were reported at the highest dose tested for lung adenomas, carcinomas or combined tumors. **In males, there was an increase in the incidence of hepatocellular adenomas at all dosed groups; however, the increase was not statistically significant. Based on the clinical signs of toxicity and the effects on ChE activity, it was determined by the HED Cancer Assessment Review Committee (CARC) that trichlorfon was tested at adequate dose levels"** (5).

Mutagenicity Studies; Trichlorfon

Trichlorfon has been examined in a wide variety of mutagenicity tests with a wide variety of responses. The summary of results from the battery of mutagenicity studies for trichlorfon are presented in Table 5. The assays include bacterial and mammalian cell cultures, with and without metabolic activation with the S9 fraction from mammalian liver microsomes. In their 1999 HED on trichlorfon toxicity chapter (5), EPA fails to specify which tests were done in vitro and which were done in whole animals. Trichlorfon has been shown positive in both bacterial and eukaryotic systems under certain conditions.

Table 5. EPA Evaluated Mutagenicity Studies for Trichlorfon (5).		
System	Activation (S9)	Results
Salmonella typhimurium	with and without	weakly positive
Saccharomyces cerevisiae	with and without	negative up to 10,000 ug/plate
Salmonella		positive at levels > 5,000 ug/plate
Escherichia coli		positive at levels > 1,000 ug/plate
in vitro mammalian cells	with and without	positive between 1 and 145 ug/ml
Unscheduled DNA synthesis	without	positive between 100 to 10,000 ug/ml
Unscheduled DNA synthesis	with	negative
DNA damage and repair in *S. typhimurium*		positive (doses not reported)
DNA damage and repair in *E coli*		negative
DNA damage and repair in *Bacillus subtilus*		negative
DNA damage and repair in *S. cerevisiae*	with and without	positive between 10,000 to 50,000 ug/ml
Sister chromatid exchange in Chinese hamster ovary cells		positive at the cytotoxic dose of 1,000 ug/ml
Sister chromatid exchange	without	positive in a dose related manner

Table 5. EPA Evaluated Mutagenicity Studies for Trichlorfon (5).		
System	Activation (S9)	Results
Sister chromatid exchange	with	inconclusive
Clastogenicity in human lymphocytes	without	positive at 3, 10 or 30 ug/ml
Recombinant DNA in *B. subtilis*		negative

Cancer summary; DDVP

Given that trichlorfon serves as a slow release formulation of DDVP, one logical question regarding the potential of trichlorfon to induce tumors in the human populations is: Are the tumors observed in the DDVP chronic bioassays similar to those seen in the trichlorfon studies?

As of 2002, EPA still classified DDVP as a group C- possible human carcinogen (31,32) using the 1986 cancer classification scheme. The most recent Office of Pesticides Programs' CARC review was done in August 1999. The committee agreed to keep the "C" classification. As a result of an inquiry following the meeting, they evaluated the database in terms of the 1999 draft Cancer Risk Assessment Guidelines (1999). Their conclusions were:

"The CARC also agreed in principle, with the Scientific Advisory Panel (SAP)'s statement "overall, the high background and variability in the incidence of this tumor, as well as its species and strain specificity, make it an invalid response for human risk assessment." Based on these conclusions and, after an informal poll of the CARC, it was determined that "suggestive" under the 1999 Draft Agency Cancer Guidelines best described the carcinogenic potential of DDVP. The rationale can be stated as follows:

- "Mononuclear cell leukemia (MCL) in the male Fischer rat has certain properties in terms of variability and reliability which limit its usefulness for human risk assessment.
- The forestomach tumors, observed at gavage doses causing inhibition of plasma and red blood cell ChE and cholinergic signs, are also limited in their use for human risk assessment.
- The fact that DDVP is only positive by the gavage route and negative by the inhalation route, which is the major route of human exposure, indicates that any classification by the oral route may be limited since localized effects in the forestomach may not be applicable to human risk assessment" (22).

The thoughts on the MCL and its relevance to human leukemia were based on: (1) the registrant's July 27, 1998, "An Evaluation of the Potential Carcinogenicity of Dichlorvos: Final Report of the Expert Panel"; (2) the report of the FIFRA SAP meeting of July 30, 1998; and (3) a memorandum of a phone conversation between Dr. Boorman of NTP and certain CARC members. Reasoning behind this conclusion include:

- MCL is common in the Fischer rat, and in the males, appears to vary in its background rate with the amount of corn oil in the animal's diet.
- The tumor type does seem to be found mainly in this Fischer strain and does not appear to be similar to leukemia in humans (adults or children).

- There was no dose response in the incidence and severity between the 2 gavage doses of 4 and 8 mg/kg/day. The overall conclusion of CARC was that, while all of this information somewhat lessened our concern, the MCL could not be totally dismissed as not being relevant to humans. This agreed with the opinion of Dr. Boorman of NTP.

Mutagenicity summary; DDVP

In 1998, an expert panel convened by SRA International, was convened to evaluate the genotoxicity of DDVP. Following a discussion of the mutagenicity database for DDVP they concluded that the weight of evidence indicates that "DDVP should not be regarded as having genotoxicity potential *in vivo*" (17). The reasons for this conclusion include:

- DDVP across 6 species, including man, favors phosphorylation over alkylation by 8 orders of magnitude,
- An absence of detectable alkylation of DNA *in vivo*,
- Data that the metabolism of DDVP eliminates or significantly reduces the genotoxicity,
- Much of the data generated through the use of extremely high doses and/or intraperitoneal route of injection to administer a compound where anticipated exposure is by inhalation, and oral/dermal exposure is irrelevant,
- DDVP is genotoxic *in vitro* in a wide variety of test systems, which are "...of minimal value in predicting either *in vivo* activity or potential risk to humans because of rapid and extensive metabolism inactivation of the compound,"
- The 1992 *in vivo* cytogenetic study (acceptable by EPA) was negative for increasing aberrant bone marrow or spermatagonial cells of treated mice at any dose level. The panel considered this finding the decisive evidence to settle both the genotoxicity concern, as well as the potential for heritable effects indicated in prior (unsatisfactory) studies,
- Genotoxicity studies with trichlorfon were consistent with the lack of DDVP genotoxicity and support the conclusions that DDVP in not genotoxic *in vivo*, and
- Results of carcinogenicity testing of DDVP, trichlorfon, and naled were consistent with the lack of DDVP genotoxicity, in declaring all three substances to be non-genotoxic carcinogens (17).

The overall conclusion of this panel were that: "DDVP does not act as a genotoxic compound *in vivo*. It is an alkylating agent only at cellular concentrations that would be lethal to intact mammalian organisms. The *in vivo* significance of the *in vitro* alkylating (DNA adduct forming) potential is biologically inconsequential under anticipated human exposure" (17).

The EPA reviewer, in general, concurred with the expert panel views. However, the reviewer recommended additional testing in the form of an *in vitro* test in a mammal cell culture system other than mouse lymphoma L5178Y to differentiate between responses of the mouse lymphoma cell line and the general mammalian response, and an *in vivo* repeat of oral cytogenetic testing in mice protected by atropine in order to assure the presence of the DDVP or an active metabolite at the target sites.

EPA Cancer Classification; Trichlorfon and DDVP

The history of EPA's Cancer Classification Systems is found in Appendix B (20). Trichlorfon has been ranked as a "Group E" (no evidence) (5) and is currently ranked as "not likely to be carcinogenic to humans at low doses, but is likely to be carcinogenic at high doses" (5, 32). DDVP is ranked as a "Group C" (possible human carcinogen) (20) and according to the March 2000 CARC report would be reclassified as "suggestive, not requiring low-dose linear extrapolation" (22). The history and the most recent (1999) EPA conclusions regarding the cancer classification of trichlorfon from the 1999 HED Human Health Effects chapter (5) are:

> "On August 31, 1994 the CPRC determined that based on the evidence presented, trichlorfon was equivocal for animal carcinogenicity. In a carcinogenicity study in Fisher 344 rats, there was an increase in the incidence of renal tubular adenomas and alveolar/ bronchiolar adenomas in males and an increase in the incidence of alveolar/ bronchiolar carcinomas in females receiving 2500 ppm of trichlorfon when these groups were compared to concurrent controls. The Committee determined trichlorfon was administered at a dose which exceeded the MTD in this study. The HED Carcinogenicity Peer Review Committee classified trichlorfon a Group E, evidence of non-carcinogenicity for humans."
>
> "On February 17, 1999, the Cancer Assessment Review Committee (CARC) evaluated additional data submitted by the registrant on the mammary gland tumors since the 1995 meeting. The CARC concluded that administration of trichlorfon was associated with increasing significant trends for mammary gland adenocarcinomas, adenoacanthomas, and combined adenomas, adenocarcinomas, and adenoacanthomas in female CD-1 mice. There was also a significant difference in the pair-wise comparison of the high-dose group with controls for mammary gland combined adenomas, adenocarcinomas, and adenoacanthomas. Additionally, the incidence was outside the historical control range. However, the highest dose was considered excessive because of significant cholinesterase inhibition and increased mortality. Also, the increase in tumor incidence was seen only at the high-dose level, there was no dose response, no decrease in latency, and there were no

precursor changes. Additionally, the CARC concurred with the previous CPRC assessment of the rat and the other mouse tumor data. ”

The Committee classified Trichlorfon as **“not likely to be carcinogenic to humans at low doses, but is likely to be carcinogenic at high doses”** (5).

Developmental and Reproduction Studies; **Trichlorfon**

In the rabbit study, pregnant rabbits received doses of 0, 10, 35 or 110 mg/kg/day on gestational days 6 to 18 via gavage. The NOAEL for maternal effects was the same as the NOAEL for developmental toxicity, 35 mg/kg/day. Maternal ChE inhibition, an increase in the number resorptions, decreased fetal body weights in males and delayed ossification of the sternebrae were observed at the LOAEL of 110 mg/kg/day (5).

In the developmental rat study, the dietary concentrations were 0, 500, 1125, 2500 ppm (equivalent to 0, 45, 102, or 227 mg/kg/day) on days 6 to 15 gestation. In this study, maternal ChE inhibition was observed at all dose levels. Effects observed in the fetuses included reduced ossification of skulls, vertebrae and sternebrae. EPA concluded that this study was unacceptable due to reporting deficiencies (5).

Developmental and Reproduction Studies; **DDVP**

Developmental studies for DDVP have been done in rats and rabbits. In the rat study, maternal toxicity (clinical signs, decreased body weight gain, decreased food consumption and feed efficiency) was observed at 21 mg/kg/day. The NOAEL for maternal toxicity was 3 mg/kg/day. The developmental NOAEL was greater than 21 mg/kg/day (HDT). ChE inhibition was not measured in this study (6).

In the rabbit study, maternal toxicity (increased mortality, and decreased body weight gain) was observed at 2.5 mg/kg/day. The LOAEL for cholinergic signs was 7 mg/kg/day. The NOAEL for maternal toxicity was 0.1 mg/kg/day. The developmental NOAEL was greater than 7 mg/kg/day (HDT). ChE inhibition was not measured in this study (6).

In the rat reproductive study, the systemic NOAEL was 2.3 mg/kg/day. The LOAEL was 8.3 mg/kg/day. Changes in estrus cycle were observed at the LOAEL. In the offspring, the NOAEL was 2.3 mg/kg/day, with a LOAEL of 8.3 mg/kg/day. Effects at the LOAEL included a decrease in the number of dams bearing litters, decrease in fertility and pregnancy indices and a decrease in pup weight (6).

Developmental Neurotoxicity Study; Trichlorfon

The Developmental Neurotoxicity Study (DNT) was completed by Bayer in 2003. The information presented below is from the abstract and conclusion sections of the Bayer report (unpublished) (10).

Pregnant Wistar rats received nominal concentrations of 0, 150, 500 or 1750 ppm technical trichlorfon in their diets from gestation day (GD) 0 to lactation day (LD) 21. The resulting weekly average doses were: 0, 13.4, 49, 145.6 mg/kg/day during gestation and 0, 33.1, 103.4 and 264.6 mg/kg/day during lactation. There were no effects on fertility index or gestation length observed at any dose (10).

Systemic maternal toxicity during lactation was observed at the mid dose and high dose levels. During gestation there were no compound related effects on maternal body weight or body weight gain. However, during lactation, 2 deaths occurred and reduced body weights was observed at the high dose. Decreases in food consumption were seen at the mid and high dose levels during the last 2 weeks of lactation. An abbreviated functional operational battery was administered during gestation and again during lactation with no compound related effects reported. ChE activity was measured on LD21, these data are presented in Table 6. (10).

Evaluations of pup related parameters were performed at birth and on postnatal day (PND) 4, PND21, PND60 and at termination of the study PND75. In the F1 generation pups there was an increase in mortality at the 1,750 concentration including deaths during lactation and post weaning. This was also reflected in a decrease in litter size at this dose level. In addition, there were lower birth weights observed at this dose level (10). At the 500 ppm concentration there were decreases in pup body weight and body weight gain at the end of lactation.

Table 6. Cholinesterase (% Control) in Dams on lactation day 21 (10).

Type ChE	Dietary Concentration (Lactation Dose)		
	150 ppm (33.1 mg/kg/day)	500 ppm (103.4 mg/kg/day)	1,750 ppm (264.6 mg/kg/day)
Brain	ns	48 %	72 %
RBC	26 %	66 %	71 %
Plasma	16 %	43 %	55 %

Necropsy results included lower body weights in the dams post lactation in the HDT. No compound related gross lesions were observed at any dose level. Neurological studies and endpoints in the offspring are summarized in Table 7.

Table 7. Summary of Neurological Tests and Endpoints for Offspring from Developmental Neurotoxicity Study for Trichlorfon in Rats (10).	
Parameter	Results
Abbreviated Field Observational Battery	Negative
Motor and locomotor activity	Increase in males and females at the high dose on PND17
Acoustical startle response	Decrease in response amplitude in both sexes at mid and high dose on PND22; No change in PND38 and PND60
Passive avoidance	Negative
Water maze	Negative
ChE inhibition	Inhibition in both sexes at the 500 ppm dose level on PND21 and 1750 ppm level on PND4 and PND21.
Brain and body weights	Decreased body weight and absolute brain weight. Relative brain weight was increased.

The NOAEL for systemic maternal effect appears to be 500 ppm, ChE was inhibited at 150 ppm. RBC ChE (26%) and brain ChE (16%) inhibition were reported at this dose (9). A decrease in food consumption and ChE inhibition RBC (66%), plasma (43%) and brain (48%) were observed at 500 ppm (103.4 mg/kg/day) on the 21st day of lactation (10).

The NOAEL for the offspring is 150 ppm (33.1 mg/kg/day (lactation dose)). Effects at the next highest dose, 500 ppm (103.4 mg/kg/day (lactation dose)) included slight ChE inhibition, decrease body weight on PND21 and a decrease in the startle response at the end of exposure, PND22 (10).

Developmental Neurotoxicity Study; DDVP

There are two DNT studies for DDVP. The primary study has been completed and is currently under review by EPA. The supplemental study is still under review by AMVAC (30). Summaries of these studies have been requested.

CLINICAL

History

Metrifonate (pesticide name trichlorfon) had been used as an anti-schistosomiasis agent in developing countries. Metrifonate was on the World Health Organization (WHO)'s essential medicine list for the treatment of Schistosomiasis until 1997 (11). It also was under development as a drug for Alzheimer's disease in the US. Selected clinical reports where kinetic information and efficacy studies with reported adverse effects are summarized below.

In clinical situations, metrifonate acts as a slow release formulation of the direct ChE inhibitor DDVP. DDVP is an irreversible inhibitor of RBC ChE, binding at the esoteric site of the enzyme. Schistosome ChE is more sensitive to inhibition by DDVP than is human ChE (12). According to the cholinergic theory of Alzheimer's Disease, some of the symptoms of the disease are the result of cortical deficiencies in cholinergic transmission (21). In both cases the mode of therapeutic action is inhibition of RBC ChE by DDVP. The stable complex formed between DDVP and AChE is reversible with oxime treatment (2-PAM) (19).

Metrifonate is still used for helminth control in developing countries, but is no longer considered the first line of defense. When used as an anti-helminthic, 3 doses (7.5 to 15 mg/kg) were administered 2 to 4 weeks apart (26). In 1999, Bayer suspended the drug approval application with US FDA (25) due to reported muscle weakness (24) and/or respiratory paralysis and problems in neuromuscular transmission (25). Imbimbo (2001), in his review of anti-ChE compounds and Alzheimer's Disease, stated that the muscle weakness observed was not considered to be cholinergic in nature, rather related to delayed neurotoxicity (21).

Pharmacokinetic/Pharmacodynamic Studies in Humans

Early studies (1990-91) investigated the pharmacokinetics of metrifonate with respect to dose levels used in the treatment of Schistosomiasis. Two pharmacokinetic studies were done in healthy volunteers. The first study examined 4 dose levels (2.5, 5, 7.5, 15 mg/kg) of metrifonate in health male volunteers (4 men per group). Plasma ChE was inhibited by 86-98% of baseline. RBC ChE was inhibited to a lesser degree (by 54 % in the high dose group) and in a dose related manner. Cholinergic side affects included nausea, vomiting, abdominal colic, diarrhea, dizziness, and headache. These side effects correlated with peak plasma level of metrifonate but not with ChE inhibition. The kinetics were linear, no differences in absorption, distribution or elimination were observed at the different dose levels (19, 27).

In another schistosomiasis pharmacokinetic study, 6 healthy male volunteers ingested 7.5 mg/kg metrifonate. Here again, plasma ChE was inhibited to a greater extent than the RBC ChE, 85 and 20% respectively. In this study both metrifonate and DDVP were measured. The plasma DDVP levels were 1% to 2% of the metrifonate levels (29).

More recent studies evaluated metrifonate in healthy human volunteers at doses (0.2 to 1.2 mg/kg/day) relevant to Alzheimer Disease treatment and measured the kinetics of RBC ChE recovery in addition to metrifonate and DDVP blood levels. As with the side effects mentioned above, the efficacy of metrifonate in alleviating Alzheimer Disease symptoms is related to the degree of AChE inhibition, rather than the blood concentrations of either the parent drug or its active metabolite, DDVP (21).

Heining and Sachse (1999) published a report (18) of two studies looking at the pharmacokinetics and the presence of food in the stomach (study I) and the timing of dose (study II). The healthy volunteers in study I, were Caucasian (12 females and 2 males) with normal body weight and ages between 50 and 68. Following a 10 hr fast, 50 mg tablets of metrifonate were administered with or with out the "American Breakfast" (22.2 g protein, 75.8 g fat, 53.5 g carbohydrate; 1,015 calories). The data collected included: metrifonate and DDVP blood levels, AChE and BChE levels at time points up to 24 hours post administration. In the second study, the volunteers were healthy Caucasians (12 males), with normal body weights, ages were 24 to 45 yrs. An 80 mg tablet of metrifonate was administered at 8:00 AM, 7:00 PM or 10:00 PM. Parameters examined were the same as in study I (18).

The conclusion of the first study was that the presence of food in the stomach reduced the rate of absorption of metrifonate, but did not alter the bioavailability of DDVP, making this food effect clinically irrelevant. The conclusion of the second study was that time of administration of metrifonate did not affect the kinetics (18).

Clinical Uses and Trials

Use of metrifonate to treat schistosomiasis dates back to 1960 (28). The traditional clinical dose used was three doses of 7.5 mg/kg administered every 2 weeks. One major problem with this regimen was individuals not returning for the 2nd or 3rd dose. In 1987, in an effort to identify an abbreviated dosing regimen that could be administered over a shorter time frame and still be efficacious, 2 studies were undertaken in Somalia. The first was a two part open study (23). The second was a randomized double blind study (28). The dose regimens evaluated in the open field trial and the incidence of adverse effects are summarized in Table 8.

Table 8. Dose Regimen and Incidence of Adverse Effects from the 1987 Open Field Trial with Metrifonate (23).

Group #	Dose Regimen	Adverse Effects
I	10 mg/kg 1 time a day for 3 days	6/7 (86%)
II	5 mg/kg 3 times a day for 1 day	0/8 (0%)
III	7.5 mg/kg 3 times a day for1 day	3/3 (100%)

The incidence of adverse effects in Groups I and III precluded administration of the third dose. The dose level from group II was used in the second part of this study. Thirty-eight persons with schistosomiasis received 5 mg/kg 3 times a day for 1 day and were followed for 4 to 6 weeks. The cure rate for the classical dosing regimen was between 40 and 80% (12). The cure rate for the abbreviated dosing regimen at 6 months was 63% and the egg reduction level was 99.2% (23).

The second study (1989) was a randomized double blind study also done in Somalia. The traditional dose regimen, 7.5 mg/kg administered every 2 weeks was compared to 5 mg/kg 3 times a day for 1 day, from the 1987 study (23). The results are summarized in Table 9. (28).

Table 9. Results of the Randomized Double Blind Efficacy Trial (1989) for Metrifonate and Schistosomiasis (28).

Dose regimen	n		Cure Rate	Egg Reduction	Adverse Effects [(a)]
	day 1	6 months			
7.5 mg/kg every 2 weeks	100	73	44	93	7 (9%)
5 mg/kg 3X a day for 1 day	101	63	40	92	9 (14%)

(a) Reported spontaneous adverse effects, most likely there was under reporting. Percentages based on the number of individuals at 6 month follow-up time point.

At this dose level, cholinergic symptoms including abdominal pain, nausea, vomiting, diarrhea, headache and vertigo were common. Atropine sulfate at 1mg every 6 hours intramuscularly took care of these symptoms without decreasing the efficacy of the drug (12).

A multi-center double blind placebo study evaluating the efficacy of metrifonate in the treatment of Alzheimer's Disease patients was published in 2000 (14). Four groups of mild to moderate Alzheimer patients (groups A, B, C, And D) received one of 4 dose regimens. Loading doses were utilized in 3 of the 4 protocols. The clinical goal was 70% inhibition of RBC AChE. These groups were pooled, increasing the power of the study. Results of the study indicate that there was no clinical benefit in the pooled low dose group. The incidence of withdrawal from the study due to adverse effects was 5.8 % in the placebo group, 9.2 % in the pooled mid dose group and 8.1 % in the high dose group (Table 10). The nature and severity of the adverse reactions were not included in the report (14).

Table 10. Summary of Adverse Effects from the Metrifonate Multi-center Double-Blind Placebo Study (14).			
Pooled Group	Dose (mg/kg/day)	n	% Adverse effects
Placebo	0	550	5.8
Low Dose	0.25	241	Not reported
Mid Dose	0.65	769	9.2
High Dose	~ 1	197	8.1

In another clinical study designed to evaluate the relative AChE inhibition in the cerebrospinal fluid (CSF), RBC and Plasma, twelve Alzheimer patients participated in 3 sequential studies. All 12 received a loading dose of 2 mg/kg/day for 5 days (this level resulted in 50 to 70% AChE inhibition in RBC) after 1 week. The group was split into a variety of groups receiving 2.9 mg/kg (0.41 mg/kg/day) for time periods ranging from 6 months to 3 years (13).

The results of the study demonstrated that inhibition of RBC AChE does not reflect inhibition of cerebral spinal fluid AChE and does not support the concept that central nervous system inhibition of AChE is the mechanism of efficacy for relief of symptoms in Alzheimer Disease patients (13). The only adverse effect reported was nausea observed in 1of 3 patients in the second leg of the study in the high dose group, resulting in a missed dose on day 2 (13).

TRICHLROFON EXPOSURE

In an effort to evaluate exposure pathways, EPA evaluates a number of scenarios. Dietary exposure, food and water are seen as both acute and chronic exposure. Residential and occupational exposures may be acute (short term duration; 1 to 7 days), subchronic (intermediate duration; 1 week to several months) or chronic (long term; lifetime; 70 yrs) depending on the label directions and the use patterns (7).

Exposure Assessment

Dietary (food)

The only food use for trichlorfon is as a pour-on use on imported cattle. EPA performed a Monte Carlo simulation using consumption levels and the Dietary Exposure Evaluation Model (DEEM^{tm}). This model uses the following inputs:

> Consumption data from the USDA Continuing Surveys of Food Intakes by Individuals 1998 to 1992 (CSFII),

Biological and Economics Analysis (BEAD) that 10.3 % of the beef/veal consumed is imported,

Levels of trichlorfon in the beef and beef products were at the re-assessed tolerance levels in cattle, (fat, 0.5 ppm; meat-by-products, 0.1 ppm; and meat 0.2 ppm), and

The conservative estimate that 100% of the imported beef was treated (6).

Single day estimates of consumption, either as a single point estimate or in a Monte Carlo simulation, were used for acute assessments. Three day averages for each sub-populations were used for chronic assessments. The sub-populations include: all infants (<1 yr), nursing infants (<1 yr), non-nursing infants (<1 yr), children (1 to 6 yrs), children (7 to 12 yrs), females (13 to 19 yrs), females (13 + yrs pregnant not nursing), males (13 to 19 yrs), and males (20 + yrs). The acute and chronic exposure results from this model at the 99.9 percentile are presented in Table 11.

Table 11. Acute Dietary Exposures from DEEM^{tm} (6).		
Population subgroup	Exposure mg/kg/day	
	Acute (99.9th percentile)	Chronic exposure
US Population (48 states)	0.001086	0.000025
All infants (< 1 yr)	0.001354	0.000011
Nursing Infants (< 1 yr)	0.001228	0.000009
Non-nursing Infants (< 1 yr)	0.001452	0.000011
Children (1 to 6 yrs)*	0.001761	0.000049
Children (7 to 12 yrs)	0.001249	0.000035
Females (13 to 19 yrs)	0.001004	0.000023
Females (13 to 19 yrs; not pregnant or nursing)	0.001004	0.000019
Males (13 to 19 yrs)	0.000971	0.000030
Males (20+ yrs)	0.000840	0.000023

As seen in Table 11, children in the 1 to 6 age groups receive the highest daily doses both from acute and chronic exposure to trichlorfon in beef and beef products.

Drinking water

In the absence of water monitoring data for trichlorfon, EPA used two models; GENEEC and SCI-GROW with worse case assumptions, highest label rate 8 lbs/acre and maximum default acreage(1 hectare, ~ 2.5 acres) to estimate high end water exposure. The agency also assumed that in the absence of treatment frequencies on the label, that 3 times a year with a 7 day re-treatment interval was realistic. Other assumptions used in the GENEEC model for surface water include 87 % of the golf courses treated and of those treated, 27% of the acreage was treated with trichlorfon (6) . The model estimates for trichlorfon residues in surface and ground water are presented in Table 12.

Table 12. EPA Expected Environmental Concentrations (EEC) Trichlorfon in ppb (6).		
Water source [Model]	Peak	Chronic
Surface water [GENEEC]	179	2.7
Ground water [SCI-Gro]	0.27	

Residential and Occupational Exposure

Trichlorfon is registered for a number of non-food agricultural sites as well as ornamental turf residential and commercial. EPA in its 2000 preliminary risk assessment evaluated four residential and 11 occupational exposure scenarios to trichlorfon in non-agricultural settings. The residential exposures were assumed to be short term (1 to 7 days) and the occupational scenarios varied from short to intermediate (21 weeks to several months) depending on the scenario (6). EPA used "*Standard Operating Procedures for Residential Exposure Assessments (Dec 1997)* and for occupational exposure. In the absence of specific trichlorfon exposure data, the *Pesticide Handlers Exposure Database Version 1.1* was used.

Residential

Residential exposure levels are calculated for dermal and inhalation exposures for residential mixers, loaders and applicators. These exposures are considered short-term (1 to 7 days) because it is not likely that a homeowner would use the product more than 7 consecutive days. A homeowner loading and applying a 6.2% granulars, wearing shorts and short sleeves, using a push spreader would receive a daily dose of 0.40053 mg/kg/day (7). These products do not require mixing. The other group of people considered in non-occupational exposures are children and adults (golfing and non-golfing) entering treated areas (Table 13) (7).

Table 13. Daily Post Application Non-Occupational Exposures from Turf Uses of Trichlorfon (7)

Exposure Scenario	Rate lbs/Acre	Duration (hrs)	Dermal Dose (mg/kg/day)
Toddler	5.4	2	0.00640
	8.2	2	0.00960
Adult	5.4	2	0.00380
	8.2	2	0.00570
Adult (golfer)	5.4	4	0.00026
	8.2	4	0.00039

Occupational

Occupational exposure assessments performed by EPA used the following inputs: label rates from 1.1 to 8.2 lb ai/acre and one of three levels of personal protective equipment (PPE): baseline PPE (long sleeve shirt, long pants, shoes and socks), minimum PPE (baseline plus, chemical resistant gloves and a respirator) or maximum PPE (minimum plus coveralls) (7).

The exposure scenarios evaluated by EPA for risk assessment purposes are:

(1) mixing/loading soluble powders for groundboom and chemigation applications;
(2) applying with groundboom equipment;
(3) mixing/loading/applying with groundboom equipment for drench application;
(4) mixing/loading/applying with high pressure handwand sprayer;
(5) mixing/loading/applying with handgun sprayer;
(6) mixing/loading/applying with low-pressure handwand sprayer;
(7) mixing/loading/applying with backpack sprayer;
(8) loading/applying with push-type drop spreader;
(9) applying granulars by sprinkler can
(10) professional lawn care operators (7).

In the evaluation of the occupational short/intermediate term risks EPA assumptions included:

- Golf course turfgrass and chemigation treatments: 40 acres;
- Turfgrass broadcast treatments: 5 acres;
- Turfgrass perimeter/spot treatments: 100 sq ft using a sprinkler can, and 1,000 ft^2 for hand-applied treatments;
- Narcissus drench treatment (groundboom): 1,000 gallons;
- Ornamental treatments: 1,000 gallons high-pressure handwand, 40 gallons for low-pressure handwand and backpack; and
- Pond/aquatic tank treatments: large pond (volume equals 15 acre-feet) and small pond (volume equals 7.5 acre-feet) (7).

EPA's exposure estimates for occupational users of trichlorfon are found in Table 14. Scenarios for label uses voluntarily canceled by Bayer are not included in Table 13.

Table 14. EPA's Calculated Exposures for Occupational Users of Trichlorfon (7).					
Scenario	Use	Exposure mg/kg/day [a]			
		Baseline [b]		PPE [c]	
		Dermal	Inhalation	Dermal	Inhalation
(1) M/L [f]; soluble powders for groundboom and chemigation applications;	Turf	17	0.2	0.769 [d]	0.040
(2) A [g]; groundboom equipment;	Turf	0.067	0.003	NA [h]	NA
(3) M/L/A; groundboom equipment for drench application;	Narcissus	0.05	0.00018	NA	NA
(4) M/L/A; high pressure handwand sprayer;	Ornamentals	ND [i]	0.027	0.526 [d]	0.005
(5) M/L/A; handgun sprayer;	Turf	ND	0.0008	0.200 [d]	NA
(6) M/L/A; low-pressure handwand sprayer; (soluble powder formulation)	Turf (spot)	ND	0.0029	0.023 [d]	NA
	Ornamentals	ND	0.009	0.071 [d]	NA
	Livestock areas	ND	0.063	0.500 [d]	0.0128
	Ponds [j]	ND	0.075 to 0.345	0.430 to 1.80 [e]	0.015 to 0.66
(7) M/L/A; backpack sprayer;	Turf (spot)	ND	0.000082	0.0067 [d]	NA

Table 14. EPA's Calculated Exposures for Occupational Users of Trichlorfon (7).					
Scenario	Use	Exposure mg/kg/day [a]			
		Baseline [b]		PPE [c]	
		Dermal	Inhalation	Dermal	Inhalation
	Ornamentals	ND	0.00026	0.01213 [d]	NA
	Livestock	ND	0.0017	0.0143 [d]	NA
(8) L/A; push-type drop spreader;	Turf Maximum rate	0.182	0.004	0.769 [d]	NA
(9) A; granulars by sprinkler can	Turf (spot)	0.0083		NA	NA
Lawn care operators [k]	Turf	0.18	0.0042	NA	NA

(a) Calculated from EPA MOEs (7)
(b) Baseline = long pants, long sleeve shirt, no gloves, open mixing and loading, open cab tractor
(c) PPE = Dust mask respirator (80 % protection factor applied to baseline)
(d) PPE = long pants, long sleeve shirt and chemical resistant gloves
(e) PPE = Double layer of clothing; chemical resistant gloves
(f) M/L = Mixer/Loader
(g) A = Applicator
(h) NA = Not Applicable
(i) ND = No Data
(j) Depending on the application rate and depth of water

(k) Exposure values determined from the Outdoor Residential Exposure Task Force (ORETF)

Another group of exposures evaluated by EPA are post application exposures to people who maintain golf courses (mowers and other maintenance) and hand-laborers attending to treated ornamentals. The registrant canceled the latter use, limiting use on ornamentals to a direct soil application. EPA believes that this limit plus a 12 hr re-entry interval (REI) will mitigate the risk from post application exposure to treated ornamentals. Golf course maintenance following treatment at 8.2 lbs ai/acre results in a daily dose of 0.00079 mg/kg/day(7).

A summary of the human exposure from clinical studies is presented in Table 15. Daily doses calculated for the highest pesticide exposures non-occupational and occupational respectively are found in Tables 16 and 17.

Table 15. Summary of Human Exposure to Trichlorfon, Clinical Uses

Scenario	Dose mg/kg/day	Duration	% Adverse effects	n
Schistosomiasis (traditional regimen)	3 doses of 7.5 mg/kg every 2 weeks	6 wks; follow-up; 6 months	7	73
Schistosomiasis (abbreviated regimen)	3 doses of 5 mg/kg in 1 day	6 wks; follow-up; 6 months	9	63
Alzheimer's Disease	0.25 mg/kg/day	10 wks	NR	241
Alzheimer's Disease	0.65 mg/kg/day	10 to 26 wks	9.2	769
Alzheimer's Disease	~1 mg/kg/day	24 wks	8.1	197

Table 16. Summary of Highest Calculated Non-occupational; Daily doses for Pesticide Uses for Human Exposure to Trichlorfon

Scenario	Dose mg/kg/day	Duration
Dietary (children 1 to 6 yrs)	0.001761	acute
Dietary (children 1 to 6 yrs)	0.000049	chronic
Post-Application; toddler	0.0069	2 hrs

Table 17. Summary of Highest Calculated Occupational (8 hrs/day); Daily doses for Pesticide Uses for Human Exposure to Trichlorfon	
Scenario	Dose mg/kg/day
M/L; soluble powders for groundboom and chemigation applications; Baseline PPE; Dermal	17
M/L/A; low-pressure handwand sprayer; (soluble powder formulation); Baseline PPE; Inhalation	0.345
Post Application Golf course maintenance; 0 days post application	0.00079

EPA's Risk Assessment Methodology

Risk of a toxic response is mathematically equal to the toxicity factor times the exposure factor. Two approaches are used by EPA OPP to estimate risk for exposure to pesticides. The Reference Dose (RfD) approach is used for dietary exposures and the Margin of Exposure (MOE) approach used for occupational and residential exposures.

The RfD is the lowest NOAEL from animals studies divided by uncertainty factors (UF) (Equation a). The uncertainty factors account for variability in the population [intraspecies, factor of 10] and extrapolation from rats to humans [interspecies, factor of 10]. Other uncertainty factors may be used at the discretion of the risk assessor. A reference dose is calculated for both acute aRfD and chronic cRfD exposures. Following the passage of the Food Quality Protection Act (FQPA) in 1996, a safety factor (between 1 and 10) [FQPA SF] for developmental toxins was included in their pesticide risk assessments based on an evaluation of the existing developmental and reproductive toxicity database. Similar to the RfDs, Population Adjusted Doses (PAD) are calculated for both acute (aPAD) and chronic (cPAD) exposures. The PAD is equal to the RfD divided by the FQPA SF (Equation b) (15). If the exposure dose (combined diet and water) exceeds the PAD, changes in registration are instituted.

Equation a: $\frac{\text{NOAEL}}{\text{UF}} = \text{RfD}$ Equation b: $\frac{\text{RfD}}{\text{FQPA SF}} = \text{PAD}$

The MOE is the NOAEL divided by the exposure dose (Equation c). EPA's level of concern (LOC) for the MOE is 100 for occupational exposures and 1000 for residential exposures (this latter value includes the FQPA SF of 10X). If the MOE is less than the level of concern, OPP requires changes in the registration and label. These changes may include cancellation, use rate reduction or addition of PPE.

Equation c: $\frac{\text{NOAEL}}{\text{Exposure dose}} = \text{MOE}$

EPA Acceptable Risk Levels for Trichlorfon

The 2000 preliminary risk assessment for trichlorfon discusses EPA's Office of Pesticides Programs' (OPP) toxicity endpoints and exposure scenarios viewed by the agency to be relevant to currently registered uses of trichlorfon. ChE inhibition is the toxic end point for all current EPA risk assessments (6).

The RfDs and PADs for trichlorfon are summarized in Table 18. These include both acute and chronic dietary (including drinking water) exposures.

Table 18. EPA Doses and Acceptable Risk Level Selections for Selected Trichlorfon Exposure Scenarios

Duration	Route	NOAEL (mg/kg/day)	RfD (mg/kg/day)	FQPA SF	Acceptable Risk Level (PAD; mg/kg/day)
Acute [(a)]	Diet	10	0.1	10	0.01
Chronic [(b)]	Diet	0.2	0.002	10	0.0002

(a) The aRfD (0.1 mg/kg/day) is equal to the NOAEL of 10 mg/kg/day from an acute rat neurotoxicity study divided by intra (factor of 10) and interspecies (factor of 10) uncertainty factors. Because this is dietary exposure the FQPA safety factor 10X is applied to calculate the aPAD. The acute PAD (0.01 mg/kg/day) is equal to the aRfD (0.1 mg/kg/day) divided by the FQPA SF (10X) (6).

(b) The cRfD (0.002 mg/kg/day) is NOAEL is 0.2 mg/kg/day from the 10 year monkey study divided intra (factor of 10) and interspecies (factor of 10) uncertainty factors. Because this is dietary exposure the FQPA safety factor 10X is applied to calculate the cPAD. The chronic PAD (0.0002 mg/kg/day) is equal to the cRfD (0.002 mg/kg/day) divided by the FQPA SF (10X) (6).

Drinking water levels of concern (DWLOC)s are calculated by EPA based on dietary exposure, default body weights and water consumption factors. Expected environmental concentrations (EEC) are then modeled and compared to the DWLOCs. The DWLOCS for acute exposure to trichlorfon are 312 ppb (mg/L) for the US population and 82 ppb (mg/L) for children 1 to 6 yrs old, the most highly exposed sub-population. The chronic DWLOCs are 6.7 ppb (mg/L) for the US population and 1.5 ppb (mg/L) for children 1 to 6 yrs old.

Evaluating residential and occupational risks using short/intermediate dermal risk assessments, EPA chose a dermal NOAEL of 100 mg/kg/day from a 21-day dermal study in rabbits. For inhalation risk assessment, they chose a NOAEL of 0.0127 mg/L (3.45 mg/kg/day) from a 21-day inhalation study in rats. No chronic residential pathways were identified by EPA (6).

EPA's target MOEs for residential and occupational exposures and when the FQPA SF is used are summarized in Table 19. Because MOEs are calculated for individual exposure scenarios they will be included in tables along with descriptions of the scenarios.

Table 19. EPA Doses and Acceptable Risk Level Selections for Selected Trichlorfon Exposure Scenarios

Duration	Route	NOAEL mg/kg	FQPA SF	Target Margin of Exposure (MOE)
Short/intermediate residential	Dermal	100	10	> 1000
Short/intermediate occupational	Dermal	100	NA	>100
Short/intermediate residential	Inhalation	3.54	10	> 1000
Short/intermediate occupational	Inhalation	3.54	NA	> 100

Summary of EPA's Risk Assessment for Trichlorfon

Dietary exposure does not exceed the aPAD (0.01 mg/kg/day) or cPAD (0.0002 mg/kg/day) (Table 17) for the US population or any of the population subgroups. Children in the age group 1 to 6 yrs old are expected to receive the highest daily doses in both the acute (0.001761 mg/kg/day) and chronic (0.000049 mg/kg/day) (Table 10). EPA's dietary risk assessment for trichlorfon is presented in Table 20.

Table 20. EPA's Dietary Risk Summary

	Dose mg/kg/day	Exposure mg/kg/day	
Duration	PAD	US Population	Children 1 to 6
Acute	0.01	0.001086	0.001761
Chronic	0.0002	0.000025	0.000049

Modeled expected environmental concentrations (EEC) and drinking water levels of concern are summarized in Table 21. Consumption of drinking water from surface water supplies exceeds EPA's DWLOC for children ages 1 to 6 yrs old (6).

Table 21. EPA DWLOCs and EECs for Surface (GENEEC) and Groundwater (SCI-GROW) for Trichlorfon in ppb						
Population	Acute			Chronic		
	DWLOC modeled	Surface water	Ground water	DWLOC modeled	Surface water	Ground water
US Population	312	179	0.27	6.1	2.7	0.27
Children Ages (1 to 6)	82	179	0.27	1.5	2.7	0.27

There are two exposure scenarios of concern, if the Board of Pesticides Control decided to change the registration status of trichlorfon from a state limited use (requiring both a license and a permit for use) to a general use homeowner product. They are residential user and post application exposure scenarios and associated risks. Another non-occupational exposure scenario evaluated by EPA was use of a golf course following treatment. The MOEs for these three exposures are provided in Table 22.

Table 22. Daily Exposures to from Turf Uses of Trichlorfon (7)	
Exposure Scenario	Combined MOE
Home owner loader and applicator using push spreader	2,400
Post application exposure Toddler	10,000 to 16,000
Post application exposure Adult	17,000 to 26,000
Post application exposure Adult (golfer)	250,000 to 380,000

The daily exposures and the assumption that EPA used are found in Tables 12 and accompanying text. The target MOEs for these exposures are 1000 (Table 19).

Assessment of occupational risk from exposure to trichlorfon is evaluated using an MOE of 100. The MOEs for the 9 occupational scenarios discussed in the EPA's TRED are summarized in Table 23.

Table 23. EPA's Calculated Exposures for Occupational Users of Trichlorfon (7).						
Scenario	MOEs [a]					
	Baseline [b]			PPE [c]		
	Dermal	Inhalation	Combined	Dermal	Inhalation	Combined
(1) M/L; soluble powders for groundboom and chemigation applications;	5.8	17	4.3	130	86	51
(2) A; groundboom equipment;	1,500	990	600	NA [d]	NA	NA
(3) M/L/A; groundboom equipment for drench application;	1,900	19,000	1,700	NA	NA	NA
(4) M/L/A; high pressure handwand sprayer;	ND [e]	130	ND	190	670	150
(5) M/L/A; handgun sprayer;	ND	4,200	ND	500	NA	450
(6) M/L/A; low-pressure handwand sprayer; (soluble powder formulation)	ND	1,200	ND	4,300	NA	NA
	ND	370	ND	1,400	NA	NA
	ND	55	ND	200	270	120
	ND	10 to 46	ND	54 to 240	52 to 230	27 to 120
(7) M/L/A; backpack sprayer;	ND	42,000	ND	15,000	NA	11,000
	ND	13,000	ND	4,700	NA	3,500
	ND	2,000	ND	700	NA	520
(8) L/A; push-type drop spreader;	550	830	330	130	NA	120

Table 23. EPA's Calculated Exposures for Occupational Users of Trichlorfon (7).						
Scenario	MOEs [a]					
	Baseline [b]			PPE [c]		
	Dermal	Inhalation	Combined	Dermal	Inhalation	Combined
(9) A; granulars by sprinkler can	12,000	1 x $10^{(6)}$	12,000	NA	NA	NA
Lawn care Operators	550	830	330			

(a) Target MOE > 100
(b) Use scenarios and PPE requirements described in Table 13.
(c) SP = Soluble powder
(d) NA = Not Applicable
(e) ND = No Data
(f) Depending on the application rate and depth of water

EPA's Risk Mitigation

The MOEs for scenario 1 (mixer and loaders for turf uses) and 6 (ponds) are lower than the target MOE of 100. EPA is proposing label changes including the use of a dust/mist respirator for individuals mixing and loading large quantities of the soluble powder formulation. The other use where the risk exceeded EPA's level of concern is ornamental ponds. This use is not on the parent labels currently registered in Maine. Two states, Arizona and Missouri, have special local needs labels for this use.

EPA's discussion of aggregate risk from trichlorfon, considering exposure from all sources from the Report on FQPA Tolerance Reassessment Progress and Interim Risk Management Decision for Trichlrofon (TRED) (7) is:

> "As noted in Chapter 3 of this TRED, the Agency's modeled acute surface water EEC exceeds the DWLOC by slightly more than a factor of two for the population subgroup, children 1-6 years old. The modeled acute surface water EEC for trichlorfon is larger than the DWLOC and therefore trichlorfon does not appear to fit within its own "risk cup." However, the Agency does not believe the currently registered uses of trichlorfon actually pose an aggregate risk concern for the general population or any population subgroup for the following reasons and trichlorfon does fit within its own risk "cup."
>
> First, predicted trichlorfon concentrations for surface water are based on a moderately refined Tier I screening model. This level of analysis is intended to identify those situations where additional information, such as monitoring data, might be needed for risk assessment and/or risk mitigation purposes. In the case of trichlorfon, the Agency believes the assessment is conservative and the EECs sufficiently small, so as not to trigger monitoring or any other data requirement to address aggregate risks based on the current use pattern.
>
> Second, trichlorfon is not registered for use in the United States on any agricultural or other dietary commodity. There is a tolerance for beef intended to cover use on cattle outside the US. The Agency's dietary assessment conservatively assumes one, tolerance level residues for all imported beef, two that all imported beef has been treated with trichlorfon, and three, that 10% of consumed beef is imported. However, it is doubtful that the most highly exposed population subgroup, children 1- 6 years old, would consume solely imported beef consistent with the conservative assumptions in the risk assessment. Additionally, the predicted surface water drinking water concentrations are based on using trichlorfon on a golf course since the Agency does not have a model scenario to quantitatively predict drinking water exposure from the residential turf use. Even though the majority of trichlorfon use is on residential turf and runoff to surface water is likely, trichlorfon's short half-life together with the expectation that not every neighborhood lawn would be treated with trichlorfon on the same day together with the mitigation measures

that will be implemented in accordance with this TRED are expected to adequately address potential surface water drinking water risks.

Lastly, non-occupational and residential risks alone are not of concern for trichlorfon. Therefore, based on the conservative trichlorfon tolerance reassessment, the Agency does not believe aggregate risks are of concern nor is confirmatory data necessary based on the current limited use patterns.

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Grub and Chafer Control
Maine BPC Medical Advisory Committee

June 29, 2005
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BACKGROUND

The following is a comparative toxicological review for grub/chafer control agents. The review has been prepared from reviews of secondary and tertiary peer reviewed literature. The types of publications include:

- EPA Registration Eligibility Documents (RED)s and background documents for REDs
- EPA's Ranking of Compounds for Carcinogenic Potential
- EPA's Integrated Risk Information System (IRIS)
- EPA Post- Food Quality Protection Act (FQPA) food tolerances
- World Health Organization (WHO) criteria documents
- Reference texts; Casarett and Doull, Handbook of Pesticide Toxicology, and Crop Protection Handbook and Goldfrank's Toxicological Emergencies
- USDA's Extension Toxicology Network (Extoxnet)
- National Library of Medicine's Hazardous Substances Data Base (HSDB)

The Food Quality Protection Act (FQPA) of 1996 set new standards for risk assessment for exposure to pesticides in the diets of infants and children. One new requirement was the creation of a developmental safety factor (FQPA SF) to be used in establishing Population Adjusted Doses (PAD) for acute (aPAD) and chronic (cPAD) exposures in food (FDCA 1996, EPA 1999b). When there is laboratory evidence that the fetus or pup is more sensitive to toxic insult by the compound, the FQPA SF remains at 10. If the data indicate no evidence of sensitivity in the developing lab animals FQPA SF may be reduced to 1.

Other requirements of the FQPA are the re-assessment of food tolerances, the safety standard of "a reasonable certainty of no harm" (FDCA 1996) and giving priority for review of pesticides which pose the highest risk (EPA 1999b). This means that pre-FQPA assessments are considerably different than post-FQPA assessments for food uses and that those active ingredients not yet assessed under the FQPA pose a lesser risk than those which have been reviewed. The organophosphates and carbamates have been or are in the process of being reviewed. The neonicotinoids are newer products and their first food uses were allowed after the FQPA. Because of this, there are FQPA level tolerance assessments for both imidacloprid and thiamethoxam. The synthetic pyrethroids are still in the queue, EPA FQPA level risk assessments will be forthcoming in the next few years and permethrin is scheduled for 2006. Pesticides with no food uses, i.e. halofenozide do not under go FQPA type assessments.

PRODUCTS

There are 25 active ingredients with active federal registrations having lawn or turf listed as sites and grubs or chafers as target pests. These compounds are identified in Appendix I. Table 1. While these compounds (Appendix I. Table 1.) are registered for the appropriate sites and pests, not all demonstrate efficacy in the New England climate. Therefore, the scope of this report is

limited to those six (6) active ingredients in grub control products determined by the University of Maine Cooperative Extension (UM CE) to have efficacy on white grubs and European chafers in Northern climates and current ornamental/lawn registrations in Maine. The exception is thiamethoxam, which is not yet registered for grubs or chafers on lawns or turf (NSPIRS 2005, SPIRS 2004, Syngenta 2004a). The chemical identifiers, efficacy on grubs and chafers and current registration status of these six compounds as of November 2004, in Maine are found in Table 1.

The group of six grub control agents encompass a wide variety of chemical classes with different mechanisms of action. There are two cholinesterase inhibitors; carbaryl and trichlorfon, carbamate and organophosphate respectively, one synthetic pyrethroid; permethrin, two neonicotinoids; imidacloprid and thimethoxam and the molting hormone agonist; halofenozide Table 1.

Mechanisms of Action

Carbaryl and trichlorfon are the two cholinesterase (ChE) inhibitors on the list of six grub/chafer control agents. Carbaryl, a carbamate is a reversible inhibitor, while trichlorfon, an organophosphate is an irreversible inhibitor of cholinesterases in the nervous system. In addition to cholinesterase inhibition some of these agents also inhibit Neurotoxic Esterase (NTE) and can cause Organophosphate Induced Delayed Neuropathy (OPDIN). The hen is the best model for this toxic effect and there are neurotoxicity studies in hens in their databases.

Permethrin is a synthetic pyrethroid. These compounds act by modifying the kinetics of gating sodium in neurons. There are two sub-classes of the synthetic pyrethroids, type I (no α cyano substitution) and type II (with α cyano substitution). Permethrin is a type I (Goldfrank 2001). The symptoms of acute poisoning in rats for type I pyrethroids include hyper-excitation, sparring, aggressiveness, enhanced startle response, whole body tremor and prostration (Klaassen 2001). The chemistry of the pyrethroids is complex with many isomers with varying toxic properties. Common technical forms of permethrin are cis: trans ratio; 40:60 pesticide use (SPIRS 2004) and the 25:75 human head lice drug use (HSDB 2004). Of the two isomers, the trans isomer is metabolized faster and the cis isomer is more acutely toxic in mammals (WHO 1990).

Imidacloprid and thimethoxam are two neonicotinoid agents. While both mammals and insects have nicotinoid receptors in the nervous system, there are different species specific sub-types. These compounds have been selected on the basis of high affinity for the insect subtype of receptor. They are also poorly absorbed and rapidly metabolized and excreted by mammals (Kreiger 2001).

Halofenozide is a diacylhydrazine molting hormone agonist. This type of compound activates the molting hormone receptor in insects and causes death by accelerating the insect molt in a lethal manner (Roberts and Hutson, 1999). Non-target species such as people, fish, and birds do not have analogous receptors or growth mechanisms.

Table 1. Lawn Care Grub Control Products to be Used in Maine Cooperative Extension List (Stewart 2003)								
Chemical Identifiers (SPIRS 2003)					Registration Status (SPIRS Feb 2005)			
Active Ingredient	Chemical Class	Mechanism	Efficacy (Stewart 2003)		ME-04/05	Use class		
			White grubs	Chafers		GUP	RUP	SLU
Carbaryl [(a)]	Carbamate	Cholinesterase inhibition	Rescue in fall; spot treatment	Rescue in fall; spot treatment; 70 to 75%	24	24	0	0
Halofenozide	Diacylhydrazine	Blocks molting	Highly, spring/ summer	Not as good as neonicotinoids; July to Aug; 56 to 100%	7	7	0	0
Imidacloprid	Neonicotinoid	Blocks nicotinic receptor in nerves	Highly, spring/ summer	Very effective; May to August; 86 to 100%	23	23	0	0
Permethrin	Pyrethroid	Affects sodium channels in nerves	Not very	?	27	20	7	0
Thiamethoxam [(b)]	Neonicotinoid	Blocks nicotinic receptor in nerves	Highly, spring/ summer	Very effective; May to Aug 91 to 100%	0	0	0	0
Trichlorfon [(c)]	Oranophosphate	Cholinesterase inhibition	Moderate; Rescue in fall	Variable; Fall average 86.2 %	5	0	0	5

(a) Carbaryl broadcast use on lawns and turf may be discontinued (EPA 2004a)

(b) No products registered for grubs or chafers on lawns or turf (NSPIRS 2005, Syngenta 2004a, SPIRS 2004).

(c) Pending the outcome of the review in Maine

ACUTE TOXICITY

The acute toxicity profiles of the six grub/chafer control active ingredients are summarized in Table 2. Supporting data and references are found in Appendix II. Tables 1 to 6. This information was developed using the technical active ingredients in all cases except for thiamethoxam. Most thiamethoxam acute toxicity data are for end use products and were obtained from the Material Safety Data Sheets (MSDS) for representative products. The oral and dermal LD_{50} s are for two end use products and the technical material.

Oral LD_{50} s in rats ranged from 163 mg/kg for trichlorfon to > 4,000 mg/kg (permethrin (with a water vehicle)) and > 5,000 mg/kg (thiamethoxam) technical products. Dermal LD_{50} s in rats were greater than or equal to 2,000 mg/kg for these materials. Inhalation toxicity levels (mg/L for 4 hrs in rats) ranged from greater than 1.09 for halofenozide to greater than 5,323 for the imidacloprid dust. One limit for the upper bounds of toxicity studies is the amount of material which can be aerosolized or suspended in the air.

With regard to dermal irritation, the materials ranked accordingly: moderate (thiamethoxam 40 % WDG, trichlorfon); slight/mild (halofenozide 1.5 G, permethrin, thiamethoxam 25% WDG) and negative (carbaryl, halofenozide 22% SC, imidacloprid, permethrin). Eye effects were rated pronounced effects (halofenozide 1.5 G, dust may cause irritation or corneal injury, permethrin conjunctivitis. There were moderate effects on the eyes with thiamethoxam 40% WDG. Carbaryl, halofenozide, halofenozide 22 SC, imidacloprid, permethrin, trichlorfon were all negative for eye effects. The grub/chafer materials were negative for dermal sensitization in guinea pigs, with the exception of trichlorfon which is a moderate contact allergen. The rankings for permethrin are from two separate reviews, Extoxnet 1996 and WHO 1990 (Appendix II. Table 5.). Differences in product specific rankings may be due to the presence of the inert ingredients in the formulations.

Under the FQPA the EPA sets acute reference doses (aRfD) and acute population adjusted doses (aPAD) in addition to the traditional chronic Reference dose (cRfD). The acute NOAELs and LOAELs (for carbaryl) for this set of compounds have been set using the acute and/or developmental neurotoxicity studies and are summarized in Table 3. Because halofenozide has no food uses and permethrin has not yet undergone FQPA tolerance reassessment there are no published acute NOAELs which have been used for establishing FQPA tolerances. A NOAEL of 20 mg/kg/day has been identified for permethrin for the 90 day feeding study (HSDB, 2004).

Table 2. Summary Acute Lethal Toxicity (from Appendix II; Tables 1 to 6)						
Study	Carbaryl	Halofenozide	Imidacloprid	Permethrin	Thiamethoxam [(a)]	Trichlorfon
Oral LD50; mg/kg	311.5♂ 302.6♀	>5000	450	430[(d)] > 4000[(e)]	> 5000	136-173
Dermal LD50 mg/kg	> 2000	> 2000	> 5,000	2000[(f)] > 4000	> 2000	2000
Inhalation LC50 mg/L	> 3.4	> 1.09	> 69[(b)] > 5323[(c)]	> 23.5	>2.56	533[(h)]
Skin irritation	Negative	Negative	Negative	Negative/Mild [(g)]	Moderate	Moderate
Eye irritation	Negative	Negative/slight	Negative	Negative/ Conjunctivitis [(g)]	Moderate	Negative
Dermal Sensitization	Negative	Not available	Negative	Negative		Moderate

(a) Data for the 40 % WDG (EPA #100-1147) (Syngenta 2004d)
(b) Aerosol
(c) Dust
(d) Oil
(e) Water
(f) No vehicle
(g) Two separate reviews (WHO 1990, Extoxnet 1996)
(h) Aerosol

Table 3. Summary Acute or Subchronic NOAELs/LOAELs Toxicity (from Appendix II, Table 7)			
AI	Study	NOAEL/LOAEL (L) mg/kg/d	Reference
Carbaryl	Developmental Neurotoxicity; rat	1	EPA, 2003a
Halofenozide	No food uses		
Imidacloprid	Acute Neurotoxicity; rat	42 (L)	EPA, 2003b
Permethrin [(a)]	90-Day diet; rat	20	HSDB, 2004
Thiamethoxam	Acute Neurotoxicity; rat	1,000	EPA, 2001
Trichlorfon	Acute Neurotoxicity; rat	10	EPA, 2000a

(a) FQPA risk assessment due in 2006; the NOAEL of 20 has not been used in setting acute reference doses for permethrin

CHRONIC TOXICITY/ CARCINOGENICITY STUDIES

Chronic toxicity studies are long term (1 or more years) or life time studies in rats, mice, dogs or monkeys. Oral chronic studies involve daily exposure using the diet, drinking water or capsules as the vehicles. In the chronic monkey study for trichlorfon the material was administered in an orange drink. EPA requires chronic studies in both sexes of at least one rodent and one non-rodent species, rat and dog preferred. The goal of the chronic studies is to determine the dose response relationship between exposure to the compound and toxic effects in the exposed animals over time. In addition, the most sensitive toxic endpoint and the No Observable Adverse Effect Level (NOAEL) are identified. Effects observed at the Lowest Observable Adverse Effect Level (LOAEL) are also noted (EPA 1998a).

Dietary carcinogenicity testing is also done in two mammalian species, usually rat and mouse (EPA 1998b). The 2 yr study in the rat may be used for evaluation of both chronic and carcinogenicity toxic endpoints (EPA 1998c). The reference dose (RfD) is an estimate of the level of exposure to a pesticide residue that is believed to have no significant harmful effects. The chronic RfD, (cRfD) accounts for daily consumption over a lifetime. If the reproductive or developmental studies provide the most sensitive endpoint, these data may be used for either the cRfD of the aRfD (EPA 1999b).

The reference dose is used for residential and occupational as well as diet and water exposures when daily exposure is expected. The FQPA SF is applied only to those scenarios where children are exposed. For example, if daily exposure were occupational, the FQPA SF would not be used. If the exposure was via diet or drinking water, it would be used. To account for these exposure scenarios where children are exposed, EPA calculates a population adjusted dose (PAD) for both chronic and acute daily exposures to children. Similar to RfD, there are chronic PAD (cPAD) and acute (aPAD) established for dietary (including drinking water) exposures (EPA 1999b).

The chronic NOAEL/LOAELs used by EPA for tolerance assessment and cancer classifications are summarized in Table 4. The particulars of the long term chronic supporting studies are found in Appendix III, Tables 1 to 6). The developmental/reproductive endpoint was used in the tolerance assessment for thiamethoxam. Data from the reproductive and developmental studies are summarized in Appendix IV, Tables 1 to 6). The post-FQPA Interim RED for carbaryl was issued by EPA in 2003 (EPA 2004a). The pre-FQPA RED for trichlorfon was issued in 1997 (EPA 1997) and the post-FQPA Tolerance RED in 2001 (EPA 2001a).

The cancer rankings are also varied, one not likely (imidacloprid), one suggestive (permethrin), one likely at high doses, not likely at low doses (trichlorfon) and two likely (carbaryl and thiamethoxam). Results of the mutagenicity studies summarized in Appendix V, Tables 1 to 6. indicate that with the exception of trichlrofon (see earlier review) the only positive results were for carbaryl in Chinese hamster ovary cells with metabolic activation and several cases at cytotoxic doses. Here again in both carcinogenicity and mutagenicity studies there are no data for halofenozide.

Table 4. Summary of Chronic NOAELS/LOAELS and Cancer Classifications (from Appendix III)				
Compound	Study	NOAEL/ LOAEL	Effects at LOAEL	Cancer Classification
Carbaryl	Dog; diet	3.1 ♀ (L)	Cholinesterase inhibition (EPA 2003 a)	EPA; old system = "C"(EPA 2004a) ; new system 'likely to be a human carcinogen' with a Q*1 = 8.75 (EPA 2004b); IARC group 3 "not classifiable" (IARC 1998)
Halofenozide	No species or dose information; excessive doses may cause methemoglobinemia target organs include blood, bone marrow and spleen and nervous systems (Dow 2004 d)			Not ranked (EPA 2004b)
Imidacloprid	Rat; diet	5.7	↓ body weight gain; ↑ thyroid lesions (EPA 2003b)	EPA; old system = E; Evidence of Non-carcinogenicity (EPA 2004b)
Permethrin	Rat; diet Dog; diet	5	↑ liver weight; ↑ liver enzymes (EPA IRIS 1992)	EPA new system: Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential (EPA 2004b); Not classifiable for carcinogenicity (HSDB 2004)
Thiamethoxam	Rat repro Mice; diet	0.6 [(a)] 2.63 ♂	testicular effects F_1 ↑ hepatocyte hypertrophy (EPA 2000a)	EPA new system: Likely to cause cancer in humans (EPA 2004b)
Trichlorfon	Monkey; orange drink	0.2	Cholinesterase inhibition (EPA 1999a)	EPA new system: Likely to carcinogenic to humans (high doses), unlikely to be carcinogenicity to humans (low doses) (EPA 2004b)

(a) Reproduction endpoint used for tolerance assessment.

SUMMARY

As would be expected when qualitatively ranking the six materials based on toxic responses, the rank of the compound differs by endpoint (Table 5.). Quantitatively, the acute and chronic RfDs, risk assessment uncertainty factors, PADs and the FQPA SF for the grub/chafer control agents are summarized in Table 6.

In comparing the 6 grub/chafer control agents molting hormone agonist, halofenozide has no food uses and therefore a limited readily available toxicity database. Four of the other five materials have FQPA level tolerances; two because they are newer materials (imidacloprid and thiamethoxam) and two because they were deemed riskier products and thereby re-registered early on in the process (carbaryl and trichlrofon). This leaves permethrin, a relatively low risk synthetic pyrethroid which is marginally efficacious product on grubs.

Of the four materials with FQPA level tolerances, the acute toxicity NOAELS used by EPA for establishing the aRfD are from the acute neurotoxicity (imidacloprid, thiamethoxam and trichlorfon) or the developmental neurotoxicity studies (carbaryl) in rats. With regard to developmental and reproductive toxicity, two materials have retained FQPA SF of 10 (thiamethoxam and trichlorfon) and the other two have been reduced to 1 (carbaryl and imidacloprid).

The cancer rankings are also varied, one not likely (imidacloprid), one suggestive (permethrin), one likely at high doses, not likely at low doses (trichlorfon) and two likely (carbaryl and thiamethoxam). Results of the mutagenicity studies summarized in Appendix V. Tables 1 to 6) indicate with the exception of trichlorfon (see earlier review) the only positive results were for carbaryl in the Chinese Hamster Ovary cell line with metabolic activation and several case where exposure was at cytotoxic dose levels. Here again in both the carcinogenicity and mutagenicity studies, no data for halofenozide was identified or reviewed.

EXPOSURE

Exposure assessments performed by EPA are formulation/application equipment dependent. The highest label use rates for either European chafers or white grubs have been used as a surrogate for exposure. These rates are expressed in terms of pounds ai/1000 ft^2. Not all labels with lawn or turf as sites and grubs or chafers as target pests were reviewed. The products were identified and sorted by formulation type and concentration. Products from each type of formulation with the higher concentrations were reviewed. The highest application rates from the labels and the efficacy study are presented in Table 7. Efficacy studies may be done at greater than label rates.

Conclusions

To be added following the MAC discussion

Table 5. Qualitative Summary; Least Toxic to Most Toxic (ND = No Data were reviewed)				
Acute oral LD_{50}	Acute NOAEL/LOAEL (L)	Dev/Repro FQPA SF	Chronic NOAEL/LOAEL (L)	Carcinogenic Rank
Thiamethoxam	Thiamethoxam	Carbaryl (1)	Imidacloprid	Imidacloprid; (E) not likely
Permethrin	Imidacloprid (L)	Imidacloprid (1)	Permethrin	Permethrin; suggestive
Halofenozide	Trichlorfon	Thiamethoxam (10)	Carbaryl (L)	Trichlorfon; likely at high doses; unlikely at low doses
Imidacloprid	Carbaryl	Trichlorfon (10)	Thiamethoxam	Carbaryl; (C) likely
Carbaryl	Permethrin ND [(a)]	Permethrin ND	Trichlorfon	Thiamethoxam; likely
Trichlorfon	Halofenozide ND	Halofenozide ND	Halofenozide ND	Halofenozide; ND

(a) ND = No data were reviewed, Data are most likely available, but review would require requesting individual studies from the registrants, some of which have not undergone EPA Peer review.

Table 6. Summary of acute (from Appendix II) and chronic (from Appendix III), NOAEL (LOAEL identified with (L), Uncertainty Factors, UF, Reference Doses (RfD), Food Quality Protection Act Safety Factors (FQPA SF) and Population Adjusted Doses (PAD)

Compound	FQPA SF	Acute				Chronic			
		NOAEL	UF	aRfD	aPAD	NOAEL	UF	cRfd	cPAD
Carbaryl	1	1	100	0.01	0.01	3.1 (L)	300	0.01	0.01
Halofenozide		No Food uses							
Imidacloprid	1	42 (L)	300	0.14	0.14	5.7	100	0.057	0.057
Permethrin	FQPA Review due in 2006					5	100	0.05	NA
Thiamethoxam	10	100	100	1	0.1	0.6	100	0.006	0.0006
Trichlorfon	10	10	100	0.1	0.01	0.2	100	0.002	0.0002

Table 7. Highest Use Rates for Ornamental and Lawns			
Compound	Formulations	lbs/1000 ft^2 (label)	lbs/Acre [(a)] (efficacy study)
Carbaryl	Emulsifiable Concentrates; 22.5 to 80 % Flowable Concentrates; 41.2 to 43 % Granulars; 1.43 to 10 % Soluble Concentrates; 23.7 % Wettable Powders (Water Soluble Packets); 80 %	0.2	10.8
Halofenozide	Emulsifiable Concentrates; 22.3 % Granulars; 0.86 to 1.5 %	0.046	2
Imidacloprid	Emulsifiable Concentrates; 21.4 % Granulars; 0.15 to 0.5 % Solution Ready to use; 0.36 to 1.47% Wettable Powders; 75 %	0.009	0.3
Permethrin	Dusts; 0.25% Emulsifiable Concentrates; 2.5 to 25.6% Granulars; 0.25 to 0.5% Solution Ready to use; 2.5%	0.02	0.272
Trichlorfon	Granulars; 6.2% Wettable Powders; 80%	0.187	10.25

(a) Efficacy studies may be done at higher than label rates.

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Appendix I.
Federal Registrations
for
Grub and Chafer Control

Table 1. Federal Registrations for Grubs or Chafers (pests) and Lawn or Turf (sites) 2003/2004 (SPIRS 2003, 2004, Stewart 2003)			
Active Ingredient	Grubs	European chafer	ME CE 2004
Azadirachtin	yes	yes	
Beauveria bassiana	yes	yes	
Bifenthrin	yes	yes	
Capsaicin	yes		
Carbaryl	yes	yes	yes
Chlorethoxfos	yes		
Chlorpyrifos	yes	yes	
Cyfluthrin	yes		
Cypermethrin	yes		
Diazinon	yes	yes	
Ethoprop	yes		
Fipronil	yes		
Tetrachlorvinphos	yes		
Halofenozide	yes	yes	yes
Imidacloprid	yes	yes	yes
Lindane	yes		
Permethrin	yes	yes	yes
Phorate	yes		
Phosmet	yes		
Potassium salts of fatty acids	yes		
Tefluthrin	yes		
Terbufos	yes		
Thiamethoxam	yes		yes
Trichlorfon	yes	yes	yes

Appendix II.
Acute Toxicity

Appendix I Table 1. Carbaryl Acute Toxicity		
Study	**Sex**	**Results**
Oral LD50; Rat (EPA 2004a)	Male	311.5 mg//kg
	Female	302.6 mg//kg
Dermal LD50 ; Rabbit (EPA 2004a)		> 2000 mg//kg
Inhalation LC50 ; Rat (EPA 2004a)		> 3.4 mg/L
Skin Irritation; Rabbit (EPA 2004a)		Negative
Eye Irritation; Rabbit (EPA 2004a)		Negative
Dermal Sensitization; Guinea Pig (EPA 2004a)		Negative
Acute Delayed Neurotoxicity; Hens (EPA 2003a)		Negative
Acute Neurotoxicity; Rat (EPA 2003a); Systemic LOAEL 10 mg/kg based on RBC, plasma and brain ChE inhibiton		

Appendix II. Table 2. Halofenozide Acute Toxicity			
Study	**1.5 Granular (Dow 2004a, 2004c)**	**22 % Soluble Concentrate (Dow 2004b, 2004d)**	**Granular (tech) (CPH 2004)**
Oral LD50 Rat	2850 mg/kg	> 5000 mg/kg	2850 mg/kg
Dermal LD50 Rat	> 2000 mg/kg	> 2000 mg/kg	> 2000 mg/kg
Inhalation LC50 Rat	> 2.7 mg/L	> 1.09 mg/L	> 2.7 m/L
Skin irritation Rabbit	Slight	Negative	Negative to slight

Appendix II. Table 2. Halofenozide Acute Toxicity			
Study	**1.5 Granular (Dow 2004a, 2004c)**	**22 % Soluble Concentrate (Dow 2004b, 2004d)**	**Granular (tech) (CPH 2004)**
Eye irritation Rabbit	Dust may cause irritation or corneal injury	Negative	Slightly irritating
Dermal Sensitization Guinea Pig	Negative	Negative	

Appendix II. Table 3. Imidacloprid Acute Toxicity	
Study	**Results**
Oral LD50 Rat (Extoxnet 2004b)	450 mg/kg
Dermal LD50 Rat (Extoxnet 2004b)	> 5,000 mg/kg
Inhalation LC50 Rat (Extoxnet 2004b)	> 69 mg/m^3 aerosol > 5323 mg/m^3 dust
Skin Irritation Rabbit (Extoxnet 2004b)	Negative
Eye Irritation Rabbit (Extoxnet 2004b)	Negative
Dermal Sensitization Guinea Pig (Extoxnet 2004b)	Negative

Appendix II. Table 4. Permethrin Acute Toxicity	
Study	**Results**
Oral LD50 Rat (WHO 1990)	430 mg/kg (oil) to > 4000 mg/kg (water) Depends on vehicle; and cis; trans ratio; Cis is the more toxic of the two isomers (WHO 1990; Extoxnet 1996)

Appendix II. Table 4. Permethrin Acute Toxicity	
Study	**Results**
Rat NOAEL (90-day diet)	20 mg/kg/day (HSDB, 2004)
Dermal LD50 Rat (WHO 1990, (Extoxnet 1996)	2000 mg/kg (no vehicle) > 4000 mg/kg
Inhalation LC50 Rat (Etoxnet 1996)	> 23.5 mg/L
Skin irritation Rabbit (WHO 1990) Rabbit (Extoxnet 1996)	negative mild
Eye irritation Rabbit (WHO 1990) Rabbit (Extoxnet 1996)	negative conjunctivitis
Dermal Sensitization Guinea Pig (WHO 1990)	negative

Appendix II. Table 5. Thiamethoxam Acute Toxicity			
Study	**Technical (CPH 2004)**	**25% WDG (Syngenta 2004d)**	**40 % WDG (Syngenta 2004e)**
Oral LD50 Rat	> 5000 mg/kg	> 5000 mg/kg	> 5000 mg/kg
Dermal LD50 Rat	> 2000 mg/kg	> 2000 mg/kg	
Dermal LD50 Rabbit			> 2000 mg/kg
Inhalation LC50 Rat			> 2.56 mg/L
Inhalation LC50 Rabbit		> 2.7 mg/L	
Skin irritation Rabbit		Slight	Moderate

Appendix II. Table 5. Thiamethoxam Acute Toxicity			
Study	**Technical (CPH 2004)**	**25% WDG (Syngenta 2004d)**	**40 % WDG (Syngenta 2004e)**
Eye irritation Rabbit		Mild	Moderate
Dermal Sensitization Guinea Pig		Negative	Not Available

Appendix II. Table 6. Trichlorfon Acute Toxicity	
Study	**Results**
Oral LD50 (EPA 1999a)	136 to 173 mg/kg
Dermal LD50 (EPA 1999a)	2,000 mg/kg
Inhalation LC50 (EPA 1999a) (RSC 1994)	533 mg/m^3 (4 hr) > 0.5 mg/L
Skin irritation v (EPA 1999a)	Moderately irritating
Eye irritation (EPA 1999a)	Non-irritating
Dermal Sensitization (EPA 1999a)	Moderate contact allergen
Acute neurotoxicity Hens (Hayes and Laws 1991)	Negative

Appendix II. Table 7. Summary Acute or Subchronic NOAELs/LOAELs Toxicity				
AI	Study	NOAEL/LOAEL (L) mg/kg/d	FQPA SF	Reference
Carbaryl	Developmental Neurotoxicity; rat	1	1	EPA, 2003a
Halofenozide	No food uses			
Imidacloprid	Acute Neurotoxicity; rat	42 (L)	1	EPA, 2003b
Permethrin [(a)]	90-Day diet; rat	20	NA	HSDB, 2004
Thiamethoxam	Acute Neurotoxicity; rat	1,000	10	EPA, 2001
Trichlorfon	Acute Neurotoxicity; rat	10	10	EPA, 2000a

(d) FQPA risk assessment due in 2006; the NOAEL of 20 has not been used in setting acute reference doses for permethrin

Appendix III.
Chronic Toxicity/Carcinogenicity

Appendix III. Table 1. Carbaryl Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Chronic Diet; doses ♂ 0, 10, 60 or 350 mg/kg/day; ♀ 0, 13, 79 or 485 mg/kg/day (EPA 2003a)				
Systemic	Male	60	350	↑Clinical signs ↓body weight ↓body weight gain ↓ Food consumption ↑ cataracts organ weight changes Non-neoplastic changes
	Female	13	79	↓body weight ↓body weight gain
ChE inhibition	Male	10	60	RBC
	Female	13	79	
	Male	10	60	Brain
	Female	13	79	
	Male	60	350	Plasma
	Female	79	485	
Carcinogenicity;	HDT:↑ benign thyroid follicular cell adenomas ♂; ↑ benign transitional cell papillomas and transitional cell carcinoma in the kidney of one male; and follicular cell carcinoma in one male ♂ HDT:↑ liver adenomas; ♀ ↑ benign transitional cell papillomas and transitional cell carcinomas ♀			
Mice Chronic Diet; doses ♂ 0, 15, 146 or 1249 mg/kg/day ♀ 0, 18, 181 or 1444 mg/kg/day (EPA 2003a)				
Systemic	Male	15	146	↑ intracytoplasmic droplets in bladder
	Female	18	181	

Appendix III. Table 1. Carbaryl Chronic Toxicity/ Carcinogenicity

Study	Sex	NOAEL	LOAEL	Effects at LOAEL
ChE inhibition	Male	15	146	RBC
	Female	181	1444	
	Male	146	1129	Brain
	Female	181	1144	
	Male	1129		Plasma
	Female	1144		
Carcinogenicity;	HDT; ↑ kidney, multiple adenomas, and carcinomas; ♂ ↑ vascular tumors (hemangiosarcomas) ♂ ↑ liver multiple adenomas, carcinomas and one hepatoblastoma; ♀ ↑ vascular tumors (hemangiosarcomas) ♀			
The HED Memo states that "the Cancer classification '*likely to be carcinogenic in humans'* was based on an increased incidence of hemangiosarcomas in male mice at all doses tested; 100, 1000 and 8000 ppm" [Dose: 15, 146 or 1249 mg/kg/day].				
Dog Diet 1yr (a) ; High dose study: ♂ and ♀ 0, 3.1, 10, 31.3 mg/kg/day Low dose study: doses ♂ 0, 0.59, 1.43, 3.83 mg/kg/day; ♀ 0, 0.64, 1.54, 4.11 mg/kg/day for 5 weeks (EPA 2003a)				
ChE inhibition	Male	1.43	3.83	Plasma
	Female	ND	3.1 (LDT)	
Cancer Rank; US EPA; old system = "C"(EPA 2004a) ; new system 'likely to be a human carcinogen' with a Q*1 = 8.75 (EPA 2004b); IARC group 3 "not classifiable" (IARC 1998); Acute RfD = 0.01 mg/kg/day, Chronic RfD = 0.01 mg/kg/day (UF = 3)				

Appendix III. Table 2. Halofenozide Chronic Toxicity/ Carcinogenicity
Study
Systemic: No species or dose information; excessive doses may cause methemoglobinemia target organs include blood, bone marrow and spleen and nervous systems (Dow 2004 d)
Cancer Rank; Not ranked (EPA 2004b)

Appendix III. Table 3. Imidacloprid Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Chronic Diet; mg/kg/day (EPA 2003b)				
Systemic	Male	5.7	16.9	↓ body weight gain ↑ thyroid lesions
	Female	7.6	24.9	
Carcinogenicity; negative				
Mice Chronic Diet; mg/kg/day (EPA 2003b)				
Systemic	Male	208	414	↓ body weight, ↓ Food consumption, ↓ water intake
	Female	274	424	
Carcinogenicity; negative				
Dog Chronic; 0 to 72 mg/kg/day (EPA 2003b)				
Systemic		72 HDT		
Cancer Classification Group E; Evidence of Non-carcinogenicity (EPA 2004b); Acute RfD = 0.14 (EPA 2003b); Chronic RFD = 0.057 (EPA 2003b), Not in EPA IRIS Data base (EPA IRIS 2004)				

Appendix III. Table 4. Permethrin Chronic Toxicity/ Carcinogenicity			
Study	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Chronic Diet; 0, 1, 5 or 25 mg/kg/day (EPA IRIS 1992)			
Systemic	5	25	↑ liver wt
Dog Chronic Diet; 0, 5, 100, ? Mg/kg/day (EPA IRIS 1992)			
Systemic	5	100	↑ liver wt ↑ liver enzymes
Cancer; Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential (EPA 2004b) Not classifiable for carcinogenicity (HSDB 2004) Chronic RfD = 0.005 mg/kg/day (EPA IRIS 1992)			

Appendix III. Table 5. Thiamethoxam Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Chronic Diet; 0, 21, 63, ? mg/kg/day ♂; 0, 50.3, 155, ? mg/kg/day ♀ (EPA 2000a)				
Systemic	Male	21	65	Lymphocytic infiltration of renal pelvis and chronic nephropathy
	Female	50.3	155	↓ body weight gain, liver and kidney lesions ↑ hemosiderosis
Carcinogenicity; negative				
Mice Chronic Diet; 0, 2.63, 63.8, ? mg/kg/day ♂; 0, 3.68, 87.6, ? mg/kg/day ♀ (EPA 2000a)				
Systemic	Male	2.63	63.8	↑ hepatocyte hypertrophy
	Female	3.68	87.6	↑ hepatocyte hypertrophy
Carcinogenicity; ↑ hepatocellular adenomas and adenocarcinomas ♂ and ♀				

Appendix III. Table 5. Thiamethoxam Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Dog Chronic Diet; 0, 4.05, 21, ? mg/kg/day ♂; 0, 4.49, 24.6, ? mg/kg/day ♀ (EPA 2000a)				
Systemic	Male	4.05	21.0	↓ ALT and atrophy in the seminiferous tubules
	Female	4.49	24.6	↑ creatinine ↓food consumption
Cancer Rank = Likely to cause cancer in humans (EPA 2004b); aRfD = 1 mg/kg/day [aPAD = 0.1 mg/kg/day]; cRfD = 0.006 mg/kg/day [cPAD = 0.0006 mg/kg/day]; (a) Population Adjusted Dose (PAD) = RfD/FQPA SF				

Appendix III. Table 6. Trichlorfon Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Chronic Diet ♂; 0, 4.4, 13.3, 75 mg/kg/day: ♀; 5.8, 17.4. 93.7 mg/kg/day (EPA 1999a)				
Systemic	Male	4.4	13.3	↑ in renal calcification ↑ in hypercholesteroemia
	Female	5.8	17.4	ChE inhibition
ChE inhibition	Male	4.4	13.3	RBC
	Female	5.8	17.4	
	Male	4.4	13.3	Brain
	Female	5.8	17.4	
Carcinogenicity;	↑ Mononuclear leukemia ♂ statistically significant; within historical range ↑ Benign pheochromocytomas ♂ Slightly out side the historical range Negative ♀			

Appendix III. Table 6. Trichlorfon Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Chronic Diet; single dose ♂ 129 mg/kg/day; ♀ 159 mg/kg/day ; exceeded the Maximum Tolerated Dose (MTD) (EPA 1999a)				
Carcinogenicity;	↑ Renal tubular adenomas ♂ not statistically significant ↑ Alveolar/bronchiolar adenomas ♂ not statistically significant ↑ Alveolar/bronchiolar carcinomas combined♀ not statistically significant **No ↑ Mononuclear leukemia or benign pheochromocytomas, which were observed in the full study**			
Dog Chronic Diet 0, 1.2, 6.3, 12.5 and 25 mg/kg/day; EPA ranked supplementary (EPA 1999a)				
ChE inhibition		6.3	12.5	RBC
Carcinogenicity; Negative				
Monkey Chronic Oral (Orange drink, vehicle) ; 0, 0.2, 1.0, 5.0 mg/kg/day (EPA 1999a)				
ChE inhibition	Male	ND	0.2	RBC
	Female	0.2	1.0	
	Male	0.2	1.0	Brain
	Female	ND	0.2	
	Male	0.2	1.0	Plasma
	Female	ND	0.2	
Carcinogenicity; Negative				
Mice Chronic Diet 0.45, 135, 405 mg/kg/day (EPA 1999a)				

Appendix III. Table 6. Trichlorfon Chronic Toxicity/ Carcinogenicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Systemic		ND	45	Clinical signs
ChE inhibition	Female	ND	45	Plasma
	Male		45	RBC
	Male		45	Brain
Carcinogenicity;	↑ Hepatocellular adenomas all doses ♂ not statistically significant ↑ Alveolar/bronchiolar adenomas ♀ statistically significant ↑ Alveolar/bronchiolar adenomas and carcinomas combined statistically significant (low and mid doses; not statistically significant at high dose ♀			
Cancer Rank; Likely to carcinogenic to humans (high doses), unlikely to be carcinogenicity to humans (low doses)				

(a) EPA ranked supplementary, (b) within historical control range, (c) ss = statistically significant, (d) slightly outside historical, range, (e) exceeded MTD, (f) ns = not statistically significant

Appendix IV. Reproductive and Developmental Toxicity Studies

Appendix IV. Table 1. Carbaryl Reproductive and Developmental Toxicity Studies				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat 3 Generation; Parental doses ♂ 5, 27, 108 mg/kg/day, ♀ 6, 31, 123 mg/kg/day; F0 ♂ 0, 5, 31 or 29 mg/kg/day, ♀ 0, 6, 36, 111 mg/kg/day and F1 ♂ 0, 6, 24, 124, ♀ 0, 6, 27 or 136 mg/kg/day (EPA 2003a)				
Parental	Male	27	108	↓body weight ↓body weight gain ↓Food consumption
	Female	31	123	
Reproduction	Male	108		HDT
	Female	123		
Offspring	Male	5	27	↑ pups with no milk in stomach ↑ mortality
	Female	6	30	
Rat Teratology Gavage; doses 0, 1, 4, or 30 mg/kg/day (EPA 2003a)				
Maternal		4	30	Clinical signs ↓body weight gain and food consumption
Offspring		4	30	↓Fetal body weight and Incomplete ossification
Rabbit Teratology Gavage; doses 0, 5, 50 or 150 mg/kg/day (EPA 2003a)				
Maternal		5	50	↓body weight gain, Plasma ChE inhibition
Offspring		50	150	↓Fetal body weight
Rat Developmental Neurotoxicity Study gavage; doses 0.0.1, 1.0, 10 mg/kg/day (EPA 2003a)				
Maternal		1	10	Clinical signs, ↓body weight gain, ↓ body weight, ChE inhibition
Offspring		1	10	Changes in brain morphometrics

Appendix IV. Table 1. Carbaryl Reproductive and Developmental Toxicity Studies				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Carbaryl FQPA SF = 1 (EPA 2004a)				

Appendix IV. Table 2. Halofenozide Reproductive and Developmental Toxicity
Study
Developmental and Reproductive Toxicity; No species or dose information; negative for birth defects; fetal toxicity seen at maternally toxic doses (Dow 2004 d)
No food uses

Appendix IV. Table 3. Imidacloprid Reproductive and Developmental Toxicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Multigenerational; 0 to 47.3 mg/kg/day (EPA 2003b)				
Parental	Male	16.5	47.3	↓ pre-mating weight gain
	Female	16.5	47.3	↓ pre-mating weight gain F1 ↓ gestational weight gain
Reproduction		47.3		HDT
Offspring		16.5	47.3	↓pup weight gain
Rat Teratology Gavage; 0, 10, 30, 100, ? mg/kg/day (EPA 2003b)				
Maternal		10	30	↓ body weight gain

Appendix IV. Table 3. Imidacloprid Reproductive and Developmental Toxicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Offspring		30	100	wavy ribs
Rabbit Teratology Gavage; 0, 24, 72, ? mg/kg/day (EPA 2003b)				
Maternal		24	72	↑ mortality ↓ body weight gain ↑ Resorptions, ↑ abortions
Offspring		24	72	↑ Skeletal abnormalities ↓ Body weight gain ↑ Resorptions
Imidacloprid FQPA Safety Factor = 1 (EPA 2003b)				

Appendix IV. Table 4. Permethrin Reproductive and Developmental Toxicity			
Study	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat 3 Generation; 0, 25, 50, 125 mg/kg/day (EPA IRIS 1992 not reviewed by WHO 1990) EPA ranked core guideline (FMC 1978)			
Parental	25	50	body tremors
Reproduction	125		HDT
Offspring		25	liver effects
Rat 3 Wistar Generation; 0, 5, 30 180 mg/kg/day (25:75) (WHO 1990)			
Parental	180		HDT
Reproduction	180		HDT
Offspring	180		HDT

Appendix IV. Table 4. Permethrin Reproductive and Developmental Toxicity			
Study	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat 3 Generation; 0, 1.06, 5.3 mg/kg/day (WHO 1990)			
Parental	5.3		HDT
Reproduction	5.3		HDT
Offspring	5.3		HDT
Rat 3 Generation; 0, 26.5, 53, 132.5 mg/kg/day (WHO 1990)			
Parental	53	132.5	Clinical signs
Reproduction	132.5		HDT
Offspring	132.5		HDT
Rat Teratology; 0 to 200 mg/kg/day (EPA IRIS 1992)			
Maternal	200 HDT		
Developmental	200 HDT		
Rabbit Teratology; 0 to 400 mg/kg/day (EPA IRIS 1992)			
Maternal	400 HDT		
Developmental	400 HDT		
Rat Teratology, Sprague Dawley, Diet; 0, 26.5 , 53, 19.5, 106, 132.5, 149, 185.5, 212 mg/kg/day (WHO 1990)			
Maternal	106	132.5	↑ placental glycogen
Developmental	212		HDT

Appendix IV. Table 4. Permethrin Reproductive and Developmental Toxicity			
Study	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Rat Teratology; Sprague Dawley, 0, 10, 20, 50 mg/kg/day (WHO 1990)			
Maternal	20	50	maternal toxicity not specified
Developmental	20	50	fetal loss and non-ossified sternebrae
Rat Teratology; Sprague Dawley, Diet, 0, 0.2, 2, 4 mg/kg/day (WHO 1990)			
Maternal	4		HDT
Developmental	4		HDT
Rat Teratology, CD, 0, 22.5, 71, 225 mg/kg/day (WHO 1990)			
Maternal	225		HDT
Developmental	225		HDT
Rat Teratology, Wistar, 0, 26.5, 53, 132.5 mg/kg/day (WHO 1990)			
Maternal	53	132.5	↑ clinical signs
Developmental	132.5		HDT
Rat Teratology, Wistar, (25:75 cis trans ratio); 0, 200 mg/kg/day (WHO 1990)			
Maternal	200		HDT
Developmental	200		HDT
Rabbit Teratology; gavage 0, 600, 1200, 1800 mg/kg/day (WHO 1990)			
Maternal		600	↓ body weight gain

Appendix IV. Table 4. Permethrin Reproductive and Developmental Toxicity			
Study	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Developmental	600	1200	embryo toxicity
Mice Teratology; IRC, 0, 15, 50, 150 mg/kg/day (WHO 1990)			
Maternal	150		HDT
Developmental	150		HDT
Permethrin FQPA Risk Assessment due 6/2006 (EPA 2004c)			

Appendix IV. Table 5. Thiamethoxam Reproductive and Developmental Toxicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Multigenerational; (EPA 2000a)				
Parental	Male	1.84	61.25	↑ kidney effects
	Female	202.06		HDT
Reproduction	Male	0.61	1.84	↑ tubular atrophy in testes F1
	Female	202.06		HDT
Offspring	Male	61.25	158.32	↓ body weight gain during lactation
	Female	79.2	202.06	
Rat Teratology Gavage; 0, 30, 200, 750, ? mg/kg/day (EPA 2000a)				
Maternal		30	200	↓ body weight, ↓ body weight gain , ↓ Food Consumption

Appendix IV. Table 5. Thiamethoxam Reproductive and Developmental Toxicity				
Study	**Sex**	**NOAEL**	**LOAEL**	**Effects at LOAEL**
Offspring		200	750	↓Pup body weight, ↑ skeletal anomalies
Rabbit Teratology Gavage; 0, 50, 150, ? (EPA 2000a)				
Maternal		50	150	↑ deaths and hemorrhages, ↓ body weight gain, ↓ Food Consumption
Offspring		50	150	↓Pup body weight gain, ↑ skeletal anomalies ↑ postimplantation loss
Thiamethoxam FQPA SF = 10 (EPA 2000a)				

Appendix IV. Table 6. Trichlorfon Chronic Reproductive and Developmental Toxicity			
Study	**NOAEL**	**LOAEL**	**LOAEL**
Rat Multigenerational; Diet 0, 15, 50, 175 mg/kg/day (EPA 1999a)			
Parental	< 15	15	ChE ↓ plasma
Reproduction	175		
Offspring	50	175	↑ dialated renal pelvises, ↓ Pup body weight
Rat Teratology Gavage; (EPA 1999a); unacceptable by EPA			
Rabbit Teratology Gavage; 0, 10, 35, 110 mg/kg/day (EPA 1999a)			
Maternal	10	35	ChE ↓ brain and RBC
Offspring	35	110	↑ resorptions, ↓ Fetal body weight, delayed ossification

Appendix IV. Table 6. Trichlorfon Chronic Reproductive and Developmental Toxicity			
Study	**NOAEL**	**LOAEL**	**LOAEL**
Rat Developmental Neurotoxicity Study gavage; gestation doses, 0. 13, 49, 146: lactation doses, 0, 33, 103, 265 mg/kg/day (Hicks 2004); doses below are the lactation doses			
Maternal	103	265	ChE ↓ brain, plasma and RBC, ↓ Food consumption
Offspring	33	103	slight ChE ↓, ↓ Pup body weight, ↓ Startle response
Trichlorfon FQPA SF = 10X (EPA 2001)			

Appendix V.
Mutagenicity Studies

Appendix V. Table 1. Carbaryl Mutagenicity Studies		
EPA Guideline Number Assay; Concentration/ Dose	**Activation (S9)**	**Results**
Carbaryl Bacterial and Mammalian Gene mutation assays EPA Guidelines (EPA 2004a)		
870.5100 Bacterial Reverse Mutation Test (EPA 1998d)		
Salmonella typhimurium 5 to 1000 ug/plate	with and without	negative
Escherichia coli 5 to 1000 ug/plate	with and without	negative
870.5385 Mammalian Bone Marrow Chromosome Aberration Test (EPA 1998i)		
Chinese Hamster Ovary cells 25 to 300 ug/ml	with	positive
Chinese Hamster Ovary cells 5 to 100 ug/ml	without	negative
Carbaryl Other Genotoxic Effects (EPA 2004a)		
870.5550 Unscheduled DNA Synthesis (UDS) in Mammalian Cells in Culture (EPA 1998f)		
UDS 0.5 to 25 ug/ml		negative
Carbaryl Special studies in mice (EPA 2004a)		
Radio labeled binding; pretreatment for two weeks at 8000 ppm (1345 mg/kg/day) followed by75 mg/kg (radio labeled)		Negative for binding; ↑microsomal protein and induction of phenobarbital metabolizing enzymes
Exposure to p53 deficient mice; 0, 1.8, 5.2, 17.5, 51.2, 164.5, or716.6 mg/kg/day for 6 months		No evidence of neoplastic or pre-neoplastic lesions in vascular tissue
Appendix V. Table 2. Halofenozide Mutagenicity Studies (No data)		

Appendix V. Table 3. Imidacloprid Mutagenicity Studies		
EPA Guideline Number **Assay; Concentration/ Dose**	**Activation (S9)**	**Results**
Imidacloprid Bacterial and Mammalian Gene mutation assays EPA Guidelines (EPA 2003b)		
870.5100 Bacterial Reverse Mutation Test (EPA 1998d)		
Bacterial Assays	with and without	negative in a battery of assays
870.5300 *In vitro* Mammalian Cell Gene Mutation Test (EPA 1998e)		
in vitro Gene mutation Mammalian in approved cell lines	with and without	negative in a battery of assays
Imidacloprid Chromosomal Aberration tests EPA Guidelines (EPA 2003b)		
870.5375 Mammalian Chromosomal Aberrations Test *in vitro* (EPA 1998l)		
in vitro Mammalian Chromosomal Aberrations Test	with and without	negative except at cytotoxic doses
870.5380 Mammalian Spermatogonial Chromosomal Aberrations Test *in vivo* (EPA 1998j)		
in vivo Mammalian Spermatogonial Chromosomal Aberrations		negative
870.5385 Mammalian Bone Marrow Chromosome Aberration Test (EPA 1998i)		
in vivo Mammalian Chromosomal Aberrations		negative
870.5395 Mammalian Erythrocyte Micronucleus Test *in vivo* (EPA 1998 k)		
in vivo Mammalian Erythrocyte Micronucleus		negative
870.5900 *In vitro* Sister Chromatid Exchange (SCE) Test (EPA 1998h)		
in vitro SCE		negative except at cytotoxic doses

Appendix V. Table 3. Imidacloprid Mutagenicity Studies		
EPA Guideline Number Assay; Concentration/ Dose	**Activation (S9)**	**Results**
Imidacloprid Other Genotoxic Effects (EPA 2003b)		
870.5550 Unscheduled DNA Synthesis (UDS) in Mammalian Cells in Culture (EPA 1998f)		
UDS		negative
870.5575 Mitotic Gene Conversion in *Saccharomyces cerevisiae* (EPA 1998g)		
in vitro mitotic Gene Conversion in *Saccharomyces cerevisiae*	with and without	negative

Appendix V. Table 4. Permethrin Mutagenicity Studies		
EPA Guideline Number [(a)] Assay; Concentration/ Dose;	**Activation (S9)**	**Results**
Salmonella typhimurium ; Ames' Assay 5 tester strains (WHO 1990)	with and without	Negative
Escherichia coli (WHO 1990)		Negative
in vitro mammalian (V79 Chinese Hamster) cells (WHO 1990)	with and without	Negative
Mouse lymphoma cells L5178Y; concentrations up to 125 ug/mL (WHO 1990)	with and without	Negative
Host mediated Assay; ICR mice and *S. typhimurium* (WHO 1990)		Negative
Clastogenicity in *Drosophila melangaster* (WHO 1990)		Negative
Chromosomal aberrations in Alderly Park rats; 0, 600, 3000 and 6000 mg/kg/day (WHO 1990)		Negative
Mouse Dominant Lethal CD mice 0, 15, 48 and 150 mg/kg (WHO 1990)		Negative

(a) The EPA FQPA Level review of Permethrin for either an RED or Food Tolerance has not been done. The RED is scheduled for 2006. Because EPA has not yet reviewed and graded the mutagenicity studies for permethrin, there are no EPA guidelines identified.

Appendix V. Table 5. Thiamethoxam Mutagenicity Studies		
EPA Guideline Number Assay; Concentration/ Dose	**Activation (S9)**	**Results**
Thiamethoxam Bacterial and Mammalian Gene mutation Assays (EPA 2000a)		
870.5100 Bacterial Reverse Mutation Test (EPA 1998d)		
Salmonella typhimurium; Ames' Assay		negative
Escherichia coli, up to 5,000 g/plate		negative
870.5265 *Salmonella typhimurium* Reverse Mutation Assay (EPA 1996)		
Salmonella typhimurium up to 5,000 g/plate	with and without	negative
870.5300 *In vitro* Mammalian Cell Gene Mutation Test (EPA 1998e)		
in vitro Gene mutation mammalian cells approved cell lines, up to solubility limit	with and without	negative
Thiamethoxam Chromosomal Aberration tests EPA Guidelines (EPA 2000a)		
870.5375 *In vitro* Mammalian Chromosomal Aberrations Test (EPA 1998l)		
in vitro Mammalian Chromosomal Aberrations		negative
870.5395 Mammalian Erythrocyte Micronucleus Test (EPA 1998 k)		
in vivo mammalian micronucleus	with and without	negative
870.5900 *In vitro* Sister Chromatid Exchange Assay (EPA 1998h)		

Appendix V. Table 5. Thiamethoxam Mutagenicity Studies		
EPA Guideline Number Assay; Concentration/ Dose	**Activation (S9)**	**Results**
In vitro Sister Chromatid Exchange	with and without	negative
Thiamethoxam Other Genotoxic Effects (EPA 2000a)		
870.5550 Unscheduled DNA Synthesis (UDS) in Mammalian Cells in Culture (EPA 1998f)		
Unscheduled DNA Synthesis		negative
Appendix V. Table 6. Trichlorfon Mutagenicity Studies		
EPA Guideline Number Assay; Concentration/ Dose;	**Activation (S9)**	**Results**
Trichlorfon Bacterial and Mammalian Gene mutation assays EPA Guidelines (EPA 1999a)		
870.5100 Bacterial Reverse Mutation Test (EPA 1998d)		
Salmonella typhimurium	with and without	weakly positive
Saccharomyces cerevisiae up to 10,000 ug/plate	with and without	negative
Salmonella at levels > 5,000 ug/plate		positive
Escherichia coli at levels > 1,000 ug/plate		positive
DNA damage and repair in *S. typhimurium* (doses not reported)		positive
DNA damage and repair in *E coli*		negative
DNA damage and repair in *Bacillus subtilus*		negative

Appendix V. Table 6. Trichlorfon Mutagenicity Studies		
EPA Guideline Number Assay; Concentration/ Dose;	**Activation (S9)**	**Results**
DNA damage and repair in *S. cerevisiae* between 10,000 to 50,000 ug/ml	with and without	positive
870.5300 *In vitro* Mammalian Cell Gene Mutation Test (EPA 1998e)		
Mammalian cells between 1 and 145 ug/ml	with and without	positive
Trichlorfon Chromosomal Aberration tests EPA Guidelines (EPA 2000a)		
870.5900 *In vitro* Sister Chromatid Exchange Assay (EPA 1998h)		
Sister chromatid exchange in Chinese hamster ovary cells (1,000 ug/ml)		positive at the cytotoxic dose
Sister chromatid exchange in a dose related manner	without	positive
Sister chromatid exchange	with	inconclusive
Trichlorfon Other Genotoxic Effects (EPA 2000a)		
870.5550 Unscheduled DNA Synthesis (UDS) in Mammalian Cells in Culture (EPA 1998f)		
Unscheduled DNA synthesis between 100 to 10,000 ug/ml	without	positive
Unscheduled DNA synthesis	with	negative
Recombinant DNA in *B. subtilis*		negative